JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 52

JANUARY 1–JUNE 15, 1936

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1936

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Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:
Entire Journal: Domestic, $3.25 a year (2 volumes).
Foreign, $4.75 a year (2 volumes).
Single numbers: Domestic, 15 cents.
Foreign, 20 cents.

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

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JOURNAL OF AGRICULTURAL RESEARCH

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ERRATA AND AUTHORS' EMENDATIONS

Page 17, line 2, "(*Saccharum officinarum* L.)" should be "(hybrids of *Saccharum officinarum* L., *S. barberi* Jes., *S. spontaneum* Jes.)."

Page 41, table 2, column 4, third line from bottom, "9.240" should be "90.240."

Page 50, line 2, "decreases" should be "increases."

Page 50, second line from bottom, "decreased" should be "increased."

Page 83, line 23, "life of many other plants" should be "life of the pollen of many other plants."

Page 123, tenth line from bottom, omit "apparently."

Opposite page 235, legend for plate 2, "optically active" should be "anisotropic."

Page 327, fourth line from bottom, "(p. 324)" should be "(p. 332)."

Page 341, last line, "330" should be "332."

Page 350, seventeenth line from bottom, "from" should be "to."

Page 497, line 13, "$M.C._1 \pm \epsilon_1$" should be "$M.C._1 \pm \epsilon$"; line 14, "$M.C._1 \pm \epsilon$" should be "$M.C._1 \pm \epsilon_1$."

Page 536, table 1, column 1, "5" should be "45."

Page 541, footnote 4, volume number given as 11 should be 2.

Page 596, legend for figure 7, "*mexican*" should be "*mericana*."

Page 633, line 15, "March 8 to May 22, 1926" should be "March 28 to May 16, 1926."

Page 730, fourth line from bottom, after "variety" add "with the exception of hydrogen ions in Valencia rinds as recorded in table 5."

Pages 732–742, tables 2, 3, 4, and 8, omit "*Percent*" in columns referring to "Flavedo-albedo ratio." Table 8, also omit "*Percent*" in column referring to "Reducing-invert sugar ratio."

Page 735, table 4, "*Pct.*" under "Hydrogen-ion concentration" should be "*pH*."

Page 744, last line, after "oranges" add "with the exception of hydrogen ions in Valencia rinds as recorded in table 5."

Pages 750–763, tables 1, 2, 3, 4, and 6, omit "*Percent*" in columns referring to "Flavedo-albedo ratio."

Pages 760 and 781, plates 1 and 2, which should appear opposite pages 760 and 781, respectively, were inadvertently omitted. These illustrations have been sent to all who receive the Journal.

Page 815, footnote 3, "and" should be "ended."

Page 826, footnote 2, should be "Collected July 8, hard-dough stage, July 18, ripe stage."

Page 833, table 12, line 5, "Double Cross 2305" should be "Double Cross 2302."

Page 875, table 9, right-hand box heading under Spray 7F, "Survival" should be "Corrected survival."

Page 967, figure 5, the value of the coordinate of the point whose abscissa is 1.4 and whose ordinate is 1.9 should be shown as 1.09.

Page 968, figure 6, the value of the coordinate of the point whose abscissa is 1.0 and whose ordinate is 1.7 should be shown as 0.76.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 52 WASHINGTON, D. C., JANUARY 1, 1936 No. 1

REACTION OF OAT VARIETIES TO PHYSIOLOGIC RACES OF LOOSE AND COVERED SMUTS OF RED OATS[1]

By GEORGE M. REED, *curator, Brooklyn Botanic Garden,* and T. R. STANTON, *senior agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Investigations by Reed (*5, 6*)[2] and Reed and Stanton (*9*) demonstrated the existence of races of loose smut, *Ustilago avenae* (Pers.) Jens., and covered smut, *U. levis* (Kell. and Sw.) Magn., that attack Fulghum and related strains of red oats. When the Fulghum oat first came into prominence, a desirable character was its supposed resistance to the smuts. Reed (*3*) carried out an extensive series of experiments with numerous varieties of oats by the use of inoculum of loose and covered smuts collected in the vicinity of Columbia, Mo. Fulghum was one of the few that stood out prominently as very resistant. These results, by means of still more extensive experiments, were confirmed by Reed, Griffiths, and Briggs (*8*). Salmon and Parker (*10*) and Stadler (*13*) also emphasized the importance of the smut resistance of Fulghum and some of its selections. However, an increasing prevalence of smut in Fulghum oats in the Southern and South Central States has been observed by the junior writer in recent years. There also is more smut in the Red Rustproof strains than formerly. Owing to the extensive culture of the Fulghum type of oats from spring seeding, loose smut is particularly common on that type in Kansas, Oklahoma, and Texas. In the Southeastern States, particularly Georgia and South Carolina, both loose and covered smuts on Fulghum are fairly common.

C. O. Johnston, associate pathologist, Division of Cereal Crops and Diseases, reported from Manhattan, Kans., on the prevalence of Fulghum smut as follows: [3]

> Loose smut continues prevalent in Texas, Oklahoma, and Kansas. Most of the smut is the form that is virulent on Fulghum. This form apparently came from the winter-sown oats of the South. With the discovery and rapid adoption of the Fulghum type of oats, this form of smut increased and rapidly moved northward, until it is now coextensive with the distribution of Fulghum. In 1934 occasional fields sown with untreated seed had as much as 25 percent of smut. Covered smut is occasionally found in Kansas and was observed on a few hybrid selections at Stillwater, Okla.

In order to obtain further information on the resistance and susceptibility of oat varieties to these new smut races, the present investigation was undertaken.

[1] Received for publication Sept. 26, 1935; issued February 1936. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Brooklyn Botanic Garden. Brooklyn Botanic Garden Contribution no. 72.

[2] Reference is made by number (italic) to Literature Cited, p. 15.

[3] JOHNSTON, C. O. U. S. Dept. Agr., Bur. Plant Indus., Div. Cereal Crops and Diseases Cereal Courier 26: 97. 1934. [Mimeographed.]

PREVIOUS INVESTIGATIONS ON PHYSIOLOGIC SPECIALIZATION OF OAT SMUTS

The discovery by Reed (*4*), in 1924, of physiologic races of oat smuts lent a new interest to the question of varietal resistance of oats. The first evidence of specialization was obtained by a comparison of loose and covered smuts from Missouri and from Wales, Great Britain. The results of Reed were confirmed by Sampson (*11*).

As soon as the existence of specialized races in the oat smuts was demonstrated, Reed (*5*) secured collections of loose smut from red oats grown in the South to determine whether specialization of the smut occurred on the red-oat group of varieties. Reed found that three collections of loose smut on Fulghum, obtained from Tennessee, Texas, and Oklahoma, very severely infected Fulghum but not Red Rustproof. Loose smut obtained on Red Rustproof from Texas infected the Red Rustproof but not the Fulghum type. Thus two distinct races of loose smut on the red oats were demonstrated.

Further data on the races adapted, respectively, to Fulghum and to Red Rustproof, as well as on other specialized races of loose and covered smuts, were published in 1929 by Reed (*6*). At that time, 11 races of loose smut and 5 races of covered smut were recognized. Sampson (*12*) published additional data on the occurrence of specialization of the oat smuts in Wales. Nicolaisen (*2*), in Germany, also has recorded data for many collections of loose smut in which he has found marked differences in reaction on oat varieties.

In 1932 Reed and Stanton (*9*) published results obtained with additional collections of loose smut on red-oat varieties. Loose smut on Norton from State College, Miss., on Kanota from Newton, Kans., and on Frazier from Lawton, Okla., gave 100-percent infection on Fulghum and high percentages of infection on Canadian and Victor, while negative results were secured with Red Rustproof and Navarro. Three collections of loose smut from Denton, Tex., and from Stillwater and Lawton, Okla., on strains of the Red Rustproof variety severely infected Red Rustproof and also Canadian; negative results were obtained on Navarro, and only a slight smutting of Victor occurred. Fulghum was resistant to two collections, while in the case of the smut from Lawton, Okla., 30 percent of the Fulghum plants were smutted. This, however, probably was due to a mixture of the two races in the original collection of spores.

The occurrence of a specialized race of covered smut that attacks Fulghum oats was recorded in the same paper (*9*). Two collections of this smut were used, one from Athens, Ga., and the other from Clemson College, S. C. Very high percentages of infection on Fulghum, Canadian, and Victor were secured with both collections, negative results being obtained with Red Rustproof and Navarro.

In 1932 Reed (*7*) demonstrated that Black Mesdag was also susceptible to the *Ustilago levis* race of Fulghum. This variety previously had manifested an unusual resistance to all hitherto known races of both loose and covered smut, and it has been widely used in oat-smut studies and hybridization experiments in different parts of the world.

IMPORTANCE OF FULGHUM OATS

During the last 15 years the Fulghum oat has become one of the most important commercial varieties in the United States. It was first reported in 1912 in southeastern Georgia, having been developed

by J. A. Fulghum, of Warrenton, Ga., who observed an exceptional plant in his field, which had been sown with Texas Rustproof. Whether this plant was a mechanical mixture or a hybrid between Red Rustproof and some other variety is not definitely known.

Fulghum is grown as a fall-sown oat in the Southern States and California. It has, however, attained economic importance in Missouri, Kansas, and Oklahoma as a spring-sown variety. Stanton (*14*), Stanton and Coffman (*15, 16*), Stanton, Love, and Down (*17*), Salmon and Parker (*10*), and Stadler (*13*) have reported on the characters and economic value of the variety and its selections, including Frazier and Kanota.

MATERIALS AND METHODS

COLLECTIONS OF LOOSE AND COVERED SMUTS

The present paper records the data for 5 collections of covered smut and 13 collections of loose smut, obtained for the most part from Fulghum and related oats. These collections were tested on more than 30 oat varieties to determine the range of specialization of the two races of smut. The older and the more recently acquired collections of both loose and covered smuts were used. The history of these collections is shown in table 1.

TABLE 1.—*History of collections of covered smut (Ustilago levis) and loose smut (U. avenae) used in tests on red oats*

Species and collection no.	Host	Collector	Locality	Year
Ustilago levis:				
11	Fulghum	T. R. Stanton	Athens, Ga.	1929
12	do	do	Clemson College, S. C.	
17	do	do	Tifton, Ga.	1932
18	do	do	do	
20	Black Mesdag	T. R. Stanton and V. C. Hubbard.	Mandan, N. Dak.	
Ustilago avenae:				
12		L. R. Hesler	Knoxville, Tenn.	1924
13	Fulghum	T. R. Stanton	Lawton, Okla.	1925
14	do	P. B. Dunkle	Denton, Tex.	
17	do	G. J. Wilds	Hartsville, S. C.	1929
18	Norton	T. R. Stanton	State College, Miss.	
19	Kanota	do	Newton, Kans.	
20	Frazier	do	Lawton, Okla.	
35	Fulghum	do	Experiment, Ga.	1932
36	do	do	Tifton, Ga.	
21	Red Rustproof	do	Denton, Tex.	1929
22	do	do	Stillwater, Okla.	
38	do	do	Experiment, Ga.	1932
39	Nortex	do	Rosslyn, Va.	

Four of the collections of covered smut were obtained in the Southeastern States by the junior writer. All of them were made from fields sown to Fulghum. *Ustilago levis*–11 and *U. levis*–12 were collected in 1929 and were used in the previous experiments. *U. levis*–17 and *U. levis*–18 were obtained in May 1932.

Ustilago levis–20 was collected on Black Mesdag by T. R. Stanton and V. C. Hubbard at the United States Northern Great Plains Field Station, Mandan, N. Dak., in August 1932. The variety was grown in an experimental plot in which the influence of smut infection on plant vigor in oats was being studied (*1*). The appearance of covered smut on Black Mesdag at Mandan is of exceptional interest. This smut from Mandan also attacked some hitherto resistant hybrid selections, of which Black Mesdag was one of the parents at the Aberdeen Substation, Aberdeen, Idaho, in 1934. These selections

were tested for the department of field crops, University of Alberta, Edmonton, Canada. The original smut used for inoculating Black Mesdag at Mandan in 1932 was a mixture of several collections of spores of *U. levis*, furnished by V. F. Tapke, Bureau of Plant Industry, United States Department of Agriculture. In view of the results of experiments reported herein, the material used evidently contained some smut spores originally collected on Fulghum.

Of the 13 collections of loose smut used in the present experiments (table 1), 9 collections, several of which were used in the earlier experiments, were from the Fulghum type of oats, and 4 were from strains of the Red Rustproof variety.

Some of the collections as originally received were mixtures, and the early tests demonstrated the fact that two or more distinct races were present. The original field of oats from which the smuts were gathered, while predominantly of one variety, evidently contained admixtures of others.

OAT SPECIES AND VARIETIES INOCULATED

In the course of all the experiments, 34 varieties or strains of oats, belonging to 6 species and subspecies of *Avena*, were used to differentiate the smut races. Definite strains of these varieties, many of them grown for many years in connection with the various experiments of the senior writer, were used. These particular strains are now recognized as of special significance in the differentiation of specialized races of loose and covered smuts of oats.

The common oat, *Avena sativa* L., was represented by 17 varieties; the common side oat, *A. sativa orientalis* (Schreb.) Alefeld, by 2 varieties; the naked oat, *A. nuda* L., by 2 varieties; and the red oat, *A. byzantina* C. Koch, by 6 varieties. In addition, one or more strains of *A. barbata* Brot., *A. brevis* Roth, *A. fatua* L., *A. strigosa* Schreb., and *A. sterilis* L. were grown.

METHODS

All of the experiments were conducted at the Brooklyn Botanic Garden in the period from 1931 to 1934. Some of the smut cultures were grown in the greenhouse, but most of them were grown in the field. The methods employed have been developed by the senior writer in connection with his various experiments with the oat smuts. After removing the hulls, the caryopses were treated for 10 minutes with a solution of formaldehyde, 1 part to 320. They were then inoculated by dusting with the dry spores, and germinated at a temperature of approximately 20° C. in sand with a low moisture content. Under these conditions the seedlings usually emerged in 4 days, and 2 to 3 days later were transplanted. The experiments with most of the varieties were repeated one or more times to insure reliable results.

EXPERIMENTAL DATA

TESTS WITH FULGHUM COLLECTIONS OF USTILAGO LEVIS

On the basis of the reaction to the five smut collections, the oat varieties are arranged in three classes: (1) Varieties susceptible to all collections; (2) varieties resistant to some collections and susceptible to others; and (3) varieties resistant to all collections. Within each of these classes the varieties are further grouped as to species.

The detailed data for the five collections of covered smut are given in table 2.

TABLE 2.—*Reaction of species and varieties of Avena to collections of Ustilago levis on Fulghum and Black Mesdag oats grown at Brooklyn*

TABLE 2.—*Reaction of species and varieties of Avena to collections of Ustilago levis on Fulghum and Black Mesdag oats grown at Brooklyn Botanic Garden, 1931–34*

Species and variety	Seed no.[1]	C. I. no.[2]	Reaction of *Avena* plants to collection [3] of—									
			U. levis–11		*U. levis*–12		*U. levis*–17		*U. levis*–18		*U. levis*–20	
			Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted
			Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
CLASS 1, SUSCEPTIBLE TO ALL COLLECTIONS												
Avena byzantina:												
Frazier	914	2381	35	45.7	71	40.8	35	77.1	37	75.6	19	84.2
Fulghum	129	3211	117	49.5	119	46.9	53	83.0	58	77.5	36	77.7
Do	1000	708	39	61.5	60	43.3	18	100.0	38	86.8	20	95.0
Kanota	906	839	78	53.8	85	52.9	50	76.0	57	68.4	20	95.0
Avena nuda:												
Hull-less	30	3007	88	14.7	86	66.2	19	84.2	36	58.3	32	81.2
Liberty	292	845	17	5.8	14	35.7	20	70.0	17	82.3	18	11.1
Avena sativa:												
Black Diamond	116	1878	58	10.3	67	53.7	20	10.0	30	13.3	34	47.0
Black Mesdag	70	1877	152	45.3	119	61.3	74	35.1	82	52.4	87	59.7
Canadian	119	1625	68	98.5	25	100.0	25	100.0	26	100.0	43	100.0
Early Champion	150	1886	90	42.2	58	79.3	18	22.2	30	26.6	30	53.3
Joanette	187	1762	75	73.3	73	89.0	18	88.8	31	51.6	35	80.0
Monarch	161	1876	110	98.1	112	97.3	49	93.8	52	96.1	52	98.0
Victor	126	1875	82	74.3	35	85.7	18	100.0	31	90.3	32	96.8
Avena sativa orientalis:												
Green Mountain	110	1892	48	37.5	73	32.8	19	57.8	26	61.5	26	65.3
CLASS 2, RESISTANT TO SOME COLLECTIONS AND SUSCEPTIBLE TO OTHERS												
Avena brevis	289	1783	71	0	59	8.4	4	0	9	0	10	0
Avena byzantina:												
Burt	175	1861	17	35.2	14	0	17	23.5	32	6.2	36	22.2
Avena sativa:												
Black Norway	118	1874	39	69.2	15	0	30	0	45	0	36	0
Danish Island	149	3210	59	1.6	38	2.6	38	0	59	0	39	7.6
Avena strigosa	587	1782	67	0	70	44.2	12	0	32	9.3	15	13.3
Avena strigosa, orcadensis intermedia Marq	590	3214	18	5.5	15	6.6	7	100.0	10	100.0	14	0

See footnotes at end of table.

TABLE 2.—*Reaction of species and varieties of Avena to collections of Ustilago levis on Fulghum and Black Mesdag oats grown at Brooklyn Botanic Garden, 1931–34*—Continued

CLASS 3, RESISTANT TO ALL COLLECTIONS

Species and variety	Seed no.[1]	C. I. no.[2]	Reaction of *Avena* plants to collection[3] of—									
			U. levis–11		*U. levis*–12		*U. levis*–17		*U. levis*–18		*U. levis*–20	
			Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted
			Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Avena byzantina:												
Navarro	939	966	60	0	36	0	18	0	38	0	16	0
Red Rustproof	999	1815	18	0	15	0	16	0	16	0	13	0
Avena fatua	908	3213	41	0	34	[4] 2.9	15	0	33	0	31	0
Avena sativa:												
Cornellian	928	1242	15	0	17	0	15	0	25	0	34	0
Danish	309	1669	55	0	37	0	15	0	37	0	36	0
Gothland	152	1898	59	0	37	0	38	0	55	0	55	0
Iogold	930	2329	19	0	19	0	17	0	16	0	16	0
Markton	752	2053	50	0	30	0	11	0	27	0	28	0
Monarch Selection	102	1879	55	0	37	0	15	0	36	0	33	0
Rossman	322	1688	57	0	39	0	19	0	38	0	34	0
Scottish Chief	124	1901	56	0	35	0	12	0	33	0	37	0
Avena sativa orientalis:												
Seizure	246	1609	56	0	39	0	18	0	34	0	36	0
Avena sterilis, macrocarpa Monsch	1002	2657	14	0	12	0	14	0	16	0	35	0

[1] Special numbers assigned to strains of these varieties inoculated and observed by the senior writer.
[2] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.
[3] *Ustilago levis*.–20, on Black Mesdag, Mandan, N. Dak. Other collections on Fulghum at following localities : *U. levis*–11, Athens, Ga.; *U. levis*–12, Clemson College, S. C.; *U. levis*–17 and –18, Tifton, Ga.
[4] Probably off-type plant or natural hybrid.

Varieties Susceptible to All Collections

Four of the collections were made on Fulghum in Georgia, Mississippi, and South Carolina, and therefore special interest is attached to the reaction of Fulghum and related varieties. In addition to two strains of Fulghum, Frazier and Kanota were inoculated. All five collections of covered smut produced smut in all of these varieties. The percentage of smutting on Fulghum (129)[4] ranged from 46.9 percent with *Ustilago levis*–12 to 83 percent with *U. levis*–17, and that on Fulghum (1000) from 43.3 percent with *U. levis*–12 to 100 percent with *U. levis*–17. Frazier gave 40.8 percent infection with *U. levis*–12 to 84.2 percent with *U. levis*–20, and Kanota 52.9 percent with *U. levis*–12 to 95 percent with *U. levis*–20.

Perhaps next in interest to the behavior of these Fulghum varieties is the fact that Black Mesdag proved susceptible to all the collections: the percentage of smutting ranged from 35.1 with *Ustilago levis*–17 to 61.3 percent with *U. levis*–12. As noted earlier, it is probable that the smut collected on Black Mesdag at Mandan, N. Dak., came originally from spores obtained in the South.

Canadian, Joanette, Monarch, and Victor gave rather high percentages of infection with all five collections. These varieties usually are susceptible to specialized races of covered smut. Monarch, in particular, has shown high susceptibility to practically all such races while being very resistant to most races of loose smut.

The varieties Black Diamond, Early Champion, Hull-less, Liberty, and Green Mountain generally gave much lower percentages of smut than the varieties just mentioned. While infections were obtained on these varieties with all the collections, the percentage varied greatly. Whether any significance may be attached to this variability has not been determined.

Varieties Resistant to Some Collections and Susceptible to Others

Burt, a red oat, gave negative results with *Ustilago levis*–12 and low percentages of smut with the other four collections. The great variability of Burt in plant characters may be paralleled by a similar variability in its reaction to the races of covered smut.

Black Norway gave negative results with four collections. However, of 39 plants inoculated with *Ustilago levis*–11, 27, or 69.2 percent, were smutted. This result seems definitely to differentiate this collection from the other four.

Danish Island gave negative results with two collections and very slight infections with *Ustilago levis*–11, *U. levis*–12, and *U. levis*–20.

A strain of *Avena brevis* gave negative results with four collections. However, 8.4 percent of the plants were infected with *Ustilago levis*–12. The smutted plants were similar to those occasionally attacked by the Missouri race of covered smut. The plants first sent up normal stems of the usual height. A few tillers infected with smut developed from the base, attaining one-half to two-thirds the height of the normal stalks.

The results with strain no. 587 of *Avena strigosa* probably are significant in separating *Ustilago levis*–12 from the other four collections,

[4] Numbers in parentheses indicate special numbers assigned to strains of these varieties inoculated and observed by George M. Reed.

since 44.2 percent of the plants were infected with *U. levis*–12, whereas negative results were secured with *U. levis*–11 and *U. levis*–17 and very low percentages of smutted plants with *U. levis*–18 and *U. levis*–20.

Intermedia, a variety of *Avena strigosa orcadensis* Marq., also has given significant results. All of the plants inoculated with *Ustilago levis*–17 and *U. levis*–18 were smutted. Infection occurred in 5.5 percent of the plants inoculated with *U. levis*–11 and in 6.6 percent of those inoculated with *U. levis*–12. *U. levis*–20 gave negative results. This variety was tested in only one series of experiments and, on account of late seeding, did not fully head, so that the number of plants in a few of the series is rather low for drawing definite conclusions.

Varieties Resistant to All Collections

Eight varieties of *Avena sativa*—Cornellian, Danish, Gothland, Iogold, Markton, Monarch Selection, Rossman, and Scottish Chief—and Seizure, a variety of *A. sativa orientalis*, proved to be resistant to all collections. It may be noted that nearly all these varieties are resistant to specialized races of covered smut while very susceptible to many races of loose smut.

Navarro and Red Rustproof, varieties of *Avena byzantina*, as well as *macrocarpa*, a variety of the wild red oat, *A. sterilis*, were resistant to all collections of *Ustilago levis*.

One strain of the common wild oat, *Avena fatua*, was resistant, although one smutted plant inoculated with *Ustilago levis*–12 was noted in the series.

TESTS WITH FULGHUM COLLECTIONS OF USTILAGO AVENAE

There were 9 collections of loose smut, 5 of which came from Tennessee, South Carolina, Mississippi, and Georgia, and 4 from Kansas, Oklahoma, and Texas. So far as definitely known, most of the collections were secured on Fulghum, while *Ustilago avenae*–20 was made on Frazier and *U. avenae*–18 on Norton, a variety originally developed from a cross in which Fulghum was one of the parents. The varieties are classified as before, i. e., on the basis of their reaction to the collections of loose smut. The data are recorded in table 3.

Varieties Susceptible to All Collections

Frazier gave a range of infection from 48.2 percent with *Ustilago avenae*–19 to 79.4 percent with *U. avenae*–18. Fulghum (129) ranged from 54.5 percent with *U. avenae*–35 to 74.3 percent with *U. avenae*–20. Fulghum (1000) ranged from 25 percent infection with *U. avenae*–35 to 95 percent with *U. avenae*–18. Kanota gave a range of 47.3 percent with *U. avenae*–35 to 70.5 percent with *U. avenae*–14. In no case were all the inoculated plants of these varieties infected.

Canadian gave high percentages of smutted plants with all of the collections. Somewhat variable results were obtained with Black Diamond, Victor, Hull-less, Liberty, and Green Mountain. Rather high percentages of infection usually were secured with most of the collections.

The strain of *Avena barbata* proved susceptible to six of the collections. Unfortunately, the number of plants was small because the seed was sown late and very poor heading resulted; although the variety was used with the other collections, no fully developed plants were secured.

TABLE 3.—*Reaction of species and varieties of Avena to collections of Ustilago avenae on Frazier, Fulghum, Kanota, and Norton oats grown at the Brooklyn Botanic Garden, 1931–34*

Species and variety	Seed no.	C. I. no.	Reaction of *Avena* plants to collection [1] of—																	
			U. avenae–12		*U. avenae*–13		*U. avenae*–14		*U. avenae*–17		*U. avenae*–18		*U. avenae*–19		*U. avenae*–20		*U. avenae*–35		*U. avenae*–36	
			Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted
			Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Avena barbata	830	2467	8	37.5	6	66.6	16	100.0			8	100.0	5	100.0	4	100.0				
Avena byzantina:																				
Frazier	914	2381	56	55.3	53	64.1	56	69.6	53	52.8	39	79.4	56	48.2	38	60.5	35	48.5	35	54.2
Fulghum	129	3211	76	55.2	74	66.2	76	73.6	73	57.5	87	63.2	95	61.0	78	74.3	55	54.5	54	70.3
Do	1000	708	39	69.2	39	87.1	37	81.0	39	71.7	20	95.0	40	57.5	38	60.5	20	25.0	20	50.0
Kanota	906	839	50	64.0	54	61.6	51	70.5	53	49.0	36	61.1	53	60.3	51	50.9	38	47.3	35	62.8
Avena nuda:																				
Hull-less	30	3007	16	56.2	17	66.6	20	80.0	17	88.2	53	86.7	38	76.3	20	25.0	18	38.8	18	83.3
Liberty	292	845	17	94.1	17	41.1	19	78.9	17	58.8	20	100.0	19	21.0	17	52.9	18	16.6	15	60.0
Avena sativa:																				
Black Diamond	116	1878	18	16.6	19	78.9	18	22.2	15	53.3	53	41.5	37	35.1	19	5.2	20	15.0	20	10.0
Canadian	119	1625	56	100.0	48	95.8	56	100.0	52	100.0	71	91.5	69	72.4	51	90.1	32	100 0	32	100.0
Victor	126	1875	57	50.8	54	57.4	53	13.2	36	83.3	78	42.3	76	42.1	54	51.8	34	44.1	35	45.7
Avena sativa orientalis:																				
Green Mountain	110	1892	18	61.1	18	83.3	17	35.2	20	75.0	57	14.0	39	7.6	18	5.5	19	21.0	19	47.3
CLASS 2, RESISTANT TO SOME COLLECTIONS AND SUSCEPTIBLE TO OTHERS																				
Avena byzantina:																				
Burt	175	1861	34	14.7	30	13.3	34	11.7	34	29.4	35	22.8	35	5.7	33	3.0	32	9.3	32	15.6
Avena fatua	908	3213	19	36.8	16	0	15	0	18	0	46	0	29	0	17	0	18	0	18	0
Avena sativa:																				
Black Norway	118	1874	17	17.6	19	21.0	18	5.5	19	21.0	37	27.0	15	6.6	19	0	18	0	18	0
Danish	309	1669	20	5.0	17	41.1	19	52.6	19	5.2	58	1.7	37	37.8	18	11.1	18	0	17	5.8
Danish Island	149	3210	20	0	18	0	19	84.2	17	5.8	59	8.4	39	17.9	19	0	20	0	18	27.7
Early Champion	150	1886	20	60.0	18	72.2	19	68.4	19	52.6	57	21.0	40	30.0	17	0	18	0	20	0
Gothland	152	1898	36	0	37	21.6	36	41.6	40	0	76	0	59	25.4	37	45.9	39	0	40	0
Monarch	161	1876	36	0	36	0	38	0	40	7.5	70	1.4	58	0	37	16.2	37	0	39	23.0
Monarch Selection	162	1879	70	0	68	82.3	73	41.0	73	0	90	0	91	61.5	68	67.6	56	19.6	54	1.8
Rossman	322	1688	19	0	15	6.6	17	41.1	18	0	57	0	40	32.5	20	15.0	20	0	20	0
Scottish Chief	124	1901	17	0	19	5.2	19	5.2	19	21.0	57	3.5	36	0	18	0	19	0	20	0
Avena sativa orientalis:																				
Seizure	246	1609	17	0	17	58.8	19	42.1	18	0	53	0	39	23.0	18	27.7	19	0	19	0
Avena strigosa orcadensis intermedia	590	3214	15	0	15	0	18	0	18	0	17	82.3	16	0	15	0	18	0	15	0

See footnotes at end of table.

TABLE 3.—*Reaction of species and varieties of Avena to collections of Ustilago avenae on Frazier, Fulghum, Kanota, and Norton oats grown at the Brooklyn Botanic Garden, 1931–34*—Continued

CLASS 3, RESISTANT TO ALL COLLECTIONS

Species and variety	Seed no.	C. I. no.	Reaction of *Avena* plants to collection [1] of—																	
			U. avenae–12		*U. avenae*–13		*U. avenae*–14		*U. avenae*–17		*U. avenae*–18		*U. avenae*–19		*U. avenae*–20		*U. avenae*–35		*U. avenae*–36	
			Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted
			Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Avena brevis	289	1783	14	0	------	------	11	0	15	0	46	0	31	0	14	0	17	0	14	0
Avena byzantina:																				
Navarro	939	966	16	0	18	0	18	0	20	0	59	0	39	0	18	0	20	0	19	0
Red Rustproof	999	1815	39	0	37	[2] 2.7	39	0	18	0	19	0	37	0	38	0	20	0	19	0
Avena sativa:																				
Black Mesdag	70	1877	18	0	14	0	18	0	16	0	57	0	35	0	18	0	19	0	19	0
Cornellian	928	1242	16	0	19	0	18	[2] 5.5	17	0	19	0	18	0	18	0	20	0	19	[2] 5.2
Iogold	930	2329	18	0	18	0	18	0	20	0	18	0	20	0	19	0	19	0	19	0
Joanette	187	1762	18	0	17	0	17	0	19	0	57	0	40	0	17	0	19	0	18	0
Markton	752	2053	19	0	19	0	19	0	20	0	52	0	37	[1] 2.7	19	0	19	0	19	0
Avena sterilis macrocarpa	1002	2657	12	0	16	0	15	0	12	0	15	0	14	0	17	0	13	0	------	------
Avena strigosa	587	1782	20	0	19	0	17	0	20	0	53	0	38	0	19	0	19	0	17	0

[1] *U. avenae*–12, host variety unknown, Knoxville, Tenn.; *U. avenae*–18, on Norton, State College, Miss.; *U. Avenae*–19, on Kanota, Newton, Kans; *U. avenae*–20, on Frazier, Lawton, Okla.; others on Fulghum at following localities: *U. avenae*–13, Lawton, Okla.; *U. avenae*–14, Denton, Tex.; *U. avenae*–17, Hartsville, S. C.; *U. avenae*–35, Experiment, Ga.; *U. avenae*–36, Tifton, Ga.

[2] Infection probably due to off-type plants or natural hybridization.

Varieties Resistant to Some Collections and Susceptible to Others

Black Norway, Monarch, and Scottish Chief (varieties of *Avena sativa*) and Burt (a variety of *A. byzantina*) gave rather variable results. The percentage of smutting usually was very low and sometimes negative. Whether any special significance may be attached to the results obtained with these varieties has not been determined.

However, the results with some other varieties indicate differences in the specialization of the collections. *Ustilago avenae*–12, *U. avenae*–17, *U. avenae*–18, *U. avenae*–35, and *U. avenae*–36, which were collected in Tennessee, Georgia, Mississippi, and South Carolina, can be separated from *U. avenae*–13, *U. avenae*–14, *U. avenae*–19, and *U. avenae*–20, collected in Kansas, Oklahoma, and Texas, by the reaction of Gothland, Monarch Selection, Seizure, and Rossman. *U. avenae*–13, *U. avenae*–14, *U. avenae*–19, and *U. avenae*–20 have given comparatively high percentages of infection on all these varieties, while low percentages or negative results have been secured with the remaining collections. The results perhaps are most striking with Monarch Selection, a large number of plants having been inoculated with the various collections of smuts. The results were entirely negative with *U. avenae*–12, *U. avenae*–17, and *U. avenae*–18, while with *U. avenae*–35, 19.6 percent of the plants, and with *U. avenae*–36, 1.8 percent were smutted. On the other hand, *U. avenae*–13 gave 82.3 percent; *U. avenae*–14, 41 percent; *U. avenae*–19, 61.5 percent; and *U. avenae*–20, 67.6 percent.

It is probable that the results with Danish Island may serve to differentiate collection *Ustilago avenae*–14, since 84.2 percent of infection was obtained. With the other collections, the results were negative or gave comparatively low percentages of infection.

It is also possible that the strain of *Avena fatua* may separate *Ustilago avenae*–12 from the other collections, since 36.8 percent of the plants were smutted, while negative results were obtained in all the other series.

Intermedia, a variety of *Avena strigosa orcadensis*, seems to separate *Ustilago avenae*–18 from the other collections, since 82.3 percent of the inoculated plants were smutted, while negative results were obtained with all the other collections.

Varieties Resistant to All Collections

Navarro and Red Rustproof, varieties of *Avena byzantina*, gave negative results with all of the collections, although one smutted plant of Red Rustproof, inoculated with *Ustilago avenae*–13, was noted. *Macrocarpa*, a variety of *A. sterilis*, gave negative results with the eight collections with which it was inoculated.

Black Mesdag, Cornellian, Iogold, Joanette, and Markton gave negative results, although smutted plants of Cornellian inoculated with *Ustilago avenae*–14 and *U. avenae*–36 and one smutted plant of Markton inoculated with *U. avenae*–19 were noted.

The strain of *Avena brevis* gave negative results with eight of the collections, no plants maturing in the series with *Ustilago avenae*–13. Strain no. 587 of *A. strigosa* also gave completely negative results.

Tests with Red Rustproof Collections of Ustilago Avenae

Previous investigations by Reed (*5*, *6*) and Reed and Stanton (*9*) demonstrated the existence of a specialized race of loose smut that

attacks Red Rustproof and closely related oat varieties. Additional data on four collections of loose smut on Red Rustproof and Nortex have been obtained. In the course of the experiments, 27 strains or varieties belonging to various species of *Avena* have been inoculated. Nearly all of these have been grown in the three series in which the smut was obtained on Red Rustproof, while seven varieties were inoculated with the smut obtained on Nortex. The data are given in table 4.

TABLE 4.—*Reaction of species and varieties of Avena to collections of Ustilago avenae on Red Rustproof oats grown at the Brooklyn Botanic Garden, 1931–34*

Species and variety	Seed no.	C. I. no.	Reaction of *Avena* plants to collection [1] of—							
			U. avenae–21		*U. avenae*–22		*U. avenae*–38		*U. avenae*–39	
			Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted	Inoculated	Smutted
			Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Avena byzantina:										
Fulghum	129	3211	40	0	20	0	20	0		
Navarro	939	966	38	0	20	0	18	0		
Nortex	907	2342			11	90.9	20	20.0	20	55.0
Red Rustproof	131	3212	17	35.2	20	25.0				
Do	265	884	20	85.0	20	50.0	18	27.7		
Do	999	1815			20	35.0	20	40.0	20	75.0
Avena brevis	289	1783	37	0	20	0				
Avena fatua	968	3213	32	96.8	25	100.0	36	77.7	19	100.0
Avena strigosa	587	1782	35	57.1	35	37.1	12	0		
Avena nuda:										
Hull-less	30	3007	39	0	19	0	19	0		
Avena sativa:										
Black Diamond	116	1878	38	2.6	16	0	19	0		
Black Mesdag	70	1877	38	0	19	0	18	0		
Canadian	119	1625	39	61.5	40	57.5	37	48.6	16	81.2
Danish	309	1669	40	0	20	0	18	0		
Danish Island	149	3210	37	2.7	20	0	19	0		
Early Champion	150	1886	36	5.5	20	5.0	18	0		
Gothland	152	1898	34	0	19	0	38	0	20	0
Joanette	187	1762	39	0	20	0	19	0		
Markton	752	2053	39	0	20	0	18	0		
Monarch	161	1876	39	0	20	0	17	0		
Monarch Selection	162	1879	39	0	19	0	17	0		
Rossman	322	1688	39	0	20	0	19	0		
Scottish Chief	124	1901	33	0	19	0	20	0		
Victor	126	1875	38	2.6	20	10.0	39	0	19	5.2
Avena sativa orientalis:										
Green Mountain	110	1892	38	0	20	0	19	0		
Seizure	246	1609	36	0	20	0	18	0		
Avena sterilis macrocarpa	1002	2657					18	0	19	15.7

[1] *U. avenae*–39 was obtained on Nortex at Rosslyn, Va. Other collections were obtained on Red Rustproof at the following localities: *U. avenae*–21, Denton, Tex.; *U. avenae*–22, Stillwater, Okla.; *U. avenae*–38, Experiment, Ga.

Strains of four varieties of *Avena byzantina* were inoculated with most of the collections. The three strains of Red Rustproof were infected by every collection with which they were inoculated. Nortex, a similar variety, also was infected with the three collections of smut with which it was inoculated. Fulghum and Navarro gave negative results.

Canadian was the only variety of *Avena sativa* severely infected, the percentages ranging from 48.6 to 81.2. The varieties Black Mesdag, Danish, Gothland, Joanette, Markton, Monarch, Monarch Selection, Rossman, and Scottish Chief gave negative results. An occasional smutted plant of Black Diamond, Danish Island, Early Champion, and Victor was observed.

Green Mountain and Seizure (varieties of *Avena sativa orientalis*), Hull-less (a variety of *A. nuda*), and the strain of *A. brevis* also gave negative results. *Macrocarpa*, a variety of *A. sterilis*, was grown in only two of the series, and in one of them three smutted plants were observed. Strain no. 587 of *A. strigosa* gave 57.1 percent infection

with *Ustilago avenae*–21 and 37.1 percent with *U. avenae*–22, while negative results were obtained with *U. avenae*–38. However, only 12 plants were grown in the experiments with the last collection.

The results obtained with *Avena fatua* are especially interesting. One strain was inoculated with all four collections, and the percentages of infection ranged from 77.7 to 100. It is evident that this strain of the species is very susceptible to the race of loose smut that attacks strains of the Red Rustproof variety.

DISCUSSION

The data recorded confirm the results of earlier investigations, indicating clearly that there are distinct races of loose and covered smuts specialized to the Fulghum type of red oats. These races, however, are not restricted to these oats but may attack varieties belonging to other species and subspecies of *Avena*.

Four collections of covered smut used in these experiments were obtained on Fulghum in Georgia and South Carolina, and one collection was obtained on Black Mesdag at Mandan, N. Dak. The evidence indicates, however, that the spores that infected Black Mesdag were introduced from the South.

Specialization within the races of *Ustilago levis*–Fulghum and *U. avenae*–Fulghum is also indicated. *U. levis*–11 is differentiated from the other collections by its ability to infect Black Norway, a variety unusually resistant to the races of covered smut. In the present experiments, 39 plants were grown and 27 (69.2 percent) were smutted. The results with strain no. 587 of *A. strigosa* probably separate *U. levis*–12 from the other four collections. Significant results with *intermedia*, a variety of *A. strigosa orcadensis*, also have been obtained. This variety has proved to be very susceptible to *U. levis*–17 and *U. levis*–18 and resistant to the other collections.

Further specialization also is indicated in the collections of loose smut. Gothland, Monarch Selection, Seizure, and Rossman appear to be susceptible to *Ustilago avenae*–13, *U. avenae*–14, *U. avenae*–19, and *U. avenae*–20, while practically negative results have been secured with the remaining collections. It is also possible that Danish Island may serve to differentiate *U. avenae*–14 from the other collections, since 84.2 percent of the plants were smutted.

The strain of *Avena fatua* was infected with only one collection of *Ustilago avenae*–Fulghum, the plants inoculated with *U. avenae*–12 giving 36.8 percent infection.

The Fulghum races of covered and loose smuts have shown very distinct differences in their reaction on the various oat varieties. Both groups severely attack the strains of Fulghum and are negative on such varieties as Navarro and Red Rustproof. Both loose and covered smuts infect severely some varieties of the *Avena sativa* group, for example, Canadian. Other varieties, however, are completely resistant to the loose smut but susceptible to the covered smut. It is especially interesting that Black Mesdag is resistant to the loose smut but susceptible to the covered smut.

The race of loose smut collected from strains of the Red Rustproof variety appears to be more limited in its capacity for infecting oat varieties. It is confined to strains of the Red Rustproof type belonging to *Avena byzantina*. Fulghum and Navarro are completely

resistant. Only one variety of the common oat, Canadian, is seriously attacked. Sporadic infections seem to occur on some other varieties, including Black Diamond, Danish Island, Early Champion, and Victor. Black Mesdag and Markton are among the varieties conspicuous for their resistance. The varieties of *A. nuda* and *A. sativa orientalis* also proved resistant. The strain of *A. brevis* was entirely free from smut. The one strain of *A. strigosa* grown contained a considerable number of smutted plants. It is especially interesting that the specific strain of *A. fatua* inoculated was fully susceptible to this race. This strain was collected in California in 1930.

SUMMARY

Five collections of *Ustilago levis* have been tested on 34 strains or varieties of oats belonging to species of *Avena*. Four collections were obtained on Fulghum in Georgia and South Carolina, and one on Black Mesdag in North Dakota. These collections proved to be highly specialized in their ability to attack certain varieties of oats. They were very similar in behavior, but minor differences, indicating the existence of subraces, were evident.

Nine collections of *Ustilago avenae*, from the southern oat-growing region, were used for inoculation experiments. Five of the collections came from Tennessee, Georgia, Mississippi, and South Carolina, and four from Kansas, Oklahoma, and Texas. These collections were similar in their reaction on many oat varieties, but some evidence was obtained indicating that specialized subraces may occur.

A distinct race of loose smut occurs on Red Rustproof oats. Canadian (a variety of *Avena sativa*) and the strains of *A. fatua* and *A. strigosa* grown in the present experiments are also susceptible.

It is noteworthy that Markton and Navarro stand out as completely resistant to all these collections of loose and covered smuts. Black Mesdag, hitherto recognized as extremely resistant to all races of smuts, was found to be decidedly susceptible to *Ustilago levis*–Fulghum, although completely resistant to *U. avenae*–Fulghum and *U. avenae*–Red Rustproof.

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CHEMICAL COMPOSITION OF JUICE FROM LOUISIANA SUGARCANE INJURED BY THE SUGARCANE BORER AND THE RED ROT DISEASE [1]

By NELSON MCKAIG, JR., *associate soil technologist, Division of Soil Fertility Investigations, Bureau of Plant Industry,* and C. A. FORT, *assistant chemist, Carbohydrate Division, Bureau of Chemistry and Soils, United States Department of Agriculture* [2]

INTRODUCTION

In a study of the effect of fertilizers and soil conditions on the composition of the juice of Louisiana sugarcane (*Saccharum officinarum* L.), unusual variations in the purity and in the nonsugars of the juice of samples were occasionally found. The fact that these samples of irregular composition appeared to be largely confined to varieties known to be highly susceptible to damage by the sugarcane borer (*Diatraea saccharalis* F.) and red rot (*Colletotrichum falcatum* Went) led to the belief that such injury had adversely affected the composition of the juice. Recently, Hinds et al. (*4*)[3] have shown that borer infestation materially reduces the purity of cane juice. They have thus confirmed the earlier work of Cross, Allbright, Van Dine, Barber, Stubbs, Morgan, and others, which has been summarized by Holloway (*5*), and which clearly indicates that both the quality and the quantity of the juice are reduced and the reducing sugars in the juice are increased by injury from borer infestation. In the original description of the red rot disease in Java, Went (*10*) presented juice analyses showing that even a light infection of the stalks by the causal fungus resulted in a significant decrease of sucrose and a corresponding increase of invert sugars. This result has also been demonstrated by Edgerton (*3*) in Louisiana. Little or no information seems available on the effect of injury to sugarcane by borers or red rot on the various nonsugars of the juice, the nonsugars being the salts and organic compounds other than sucrose and reducing sugars. Therefore, analyses of the juice of sound and of injured canes have been made to determine whether borer and red rot injury had the same effect on the nonsugars as on the carbohydrate fraction of the juice. As a result of these investigations, reported herein, previous observations on the effect of borer and red rot injury on extraction and juice purity are confirmed, and further data have been obtained which indicate that these injuries materially alter the quantity and composition of the nonsugars in the juice.

MATERIALS AND METHODS

On November 27, 1931, during the harvest of certain experimental plots of plant sugarcane grown on Sharkey silty clay at the United States Sugar Plant Field Station near Houma, La., a number of

[1] Received for publication Sept. 9, 1935; issued February 1936.

[2] The writers gratefully acknowledge the cooperation of E. V. Abbott, Division of Sugar Plant Investigations, Bureau of Plant Industry, who collected the samples of sugarcane used in this investigation and estimated the degree of red rot and sugarcane borer injury.

[3] Reference is made by number (italic) to Literature Cited, p. 24.

apparently equally mature mill-length stalks of the four varieties shown in table 1 were collected, split lengthwise, and examined for borer and red rot injury. From this material three samples of each variety were selected: (1) Wholly sound stalks, (2) stalks with an approximately equal amount of borer injury but free from red rot, and (3) stalks showing approximately uniform amounts of both red rot and borer damage. Each sample thus obtained consisted of about 10 stalks of cane. The percentages of infestation were recorded at the time of collection of the samples and are expressed as percentages of internodes affected. Immediately after collection the samples were crushed in a hydraulically equipped sugarcane mill and the juice freed of bagassillo, sediment, and foam by a standardized procedure developed by the writers. The resulting "standard raw crusher juice" was analyzed for juice solids with Brix spindles and for apparent sucrose by direct polarization of the undiluted juice after clarification with dry basic lead acetate (*9*). Acidity was determined by titration of 10 cc of juice, after dilution with distilled water, with 0.1 N alkali, using phenolphthalein as indicator. The total nitrogen was determined by the Gunning method (*2*), protein nitrogen by precipitation with tannic acid, and analysis of the precipitate for nitrogen according to the micro-Kjeldahl method of Pregl (*7*). Gums were estimated by acid hydrolysis of the alcohol-insoluble precipitate formed by mixing 1 volume of juice with 7 volumes of 95-percent ethyl alcohol and determination of the liberated reducing sugars. The ash was determined by evaporation of a known quantity of juice and subsequent ignition of the sirup in an electric muffle at a temperature below visible redness. With the exception of chloride, which was estimated on a separate portion of juice by a modification of Pettinger's method (*6*), the individual mineral constituents of the ash were determined by analysis of the ash solution after removal of silica.

In addition to the raw-juice analyses, the juice from the series of samples from the two varieties P. O. J. 213 and P. O. J. 36–M was given a standardized sugar-house clarification. The sulphitation process of clarification was employed, with 0.6 g of sulphur dioxide per liter of juice, the juice being limed cold to such a degree that the clarified juice, after being brought to a boil and settling, had a pH of 6.8 to 7.0. Evaporation of the clarified juice to an effect sirup density of 55° Brix was conducted in a Pyrex glass vacuum pan. These sirups were then analyzed, and thus the corresponding composition of the samples at the stage at which they would be used for boiling to sugar was obtained. The analyses included the determination of true purity (by the Clerget method), reducing sugars, ash, alcohol precipitate, gums, total nitrogen, and the four ash constituents silica, lime, sulphur, and iron. Color and turbidity were estimated by means of a Keuffel & Esser color analyzer. The observed percentage transmission at 560μ was calculated to $-\log t$ values.

On October 31, 1932, a second set of samples of P. O. J. 213 plant cane was collected from a different location on the same soil type at the field station, and on November 3, samples of Co. 281 plant cane were collected from an adjacent plot. These canes differed from those collected in 1931 in that they consisted of the lower 10 joints only. The samples of sound cane showed no evidence of borer

or of red rot; the diseased stalks were extensively damaged by the borer and every joint showed the characteristic red coloration of the red rot fungus. The milling and analytical procedures used on the raw juices were the same as in 1931, except that the acidity was determined by electrometric titration to pH 7.0.

These samples were also clarified by a factory method, this time lime alone being used to yield a clarified juice of pH 6.8 to 7.0. The clarified juice was not boiled to a sirup, but was analyzed for true purity, reducing sugars, ash, and turbidity.

EXPERIMENTAL RESULTS

TESTS IN 1931

The data of table 1 fully confirm previous work and show that borer infestation alone reduces the soluble solids, sucrose, and apparent purity of crusher juice below those of comparable sound cane and that the juice extraction is decreased as a result of the injury. The decreased sucrose and purity values indicate losses of available 96° sugar, ranging from 7.9 pounds to over 31 pounds per ton of cane. These values were calculated by the use of the Winter-Carp formula on the assumption of uniform extraction and reduction factors comparable to those obtained commercially (*8*); if the values obtained were corrected for the decreased extraction incident to the injury, the apparent losses would be greater. When borer damage is accompanied by red rot, the observations indicate that still further reduction in quality of juice may be expected. As further shown in table 1, the combination of borer and red rot injury, to the extent shown, reduces the solids and purity of the juice to values lower than if borer infestation alone were present, except in the case of P. O. J. 36–M. The indicated sugar losses ranged from 40.7 to over 71 pounds of sugar per ton of cane when not corrected for the extraction differences indicated in the table.

TABLE 1.—*Effect of borer and red rot injury on the quantity and quality of crusher juice from 4 varieties of sugarcane, United States Sugar Plant Field Station, Houma, La., 1931*

Variety	Internodes injured by borer	Internodes injured by red rot	Brix	Apparent sucrose	Apparent purity	Indicated 96° sugar per ton of cane		Cane for 1 ton of sugar	Crusher juice extraction
						Total	Loss due to injury		
	Percent	*Percent*	*Degrees*	*Percent*	*Percent*	*Pounds*	*Pounds*	*Tons*	*Percent*
P. O. J. 36–M	0.0	0.0	16.66	14.09	84.57	191.8	-------	10.43	69.3
	24.2	.0	15.41	12.22	79.30	160.2	31.6	12.48	68.6
	47.5	32.5	13.86	10.24	73.88	128.4	63.4	15.58	67.4
P. O. J. 213	.0	.0	16.96	14.58	85.97	200.1	-------	10.00	71.6
	33.3	.0	15.76	13.19	83.69	178.4	21.7	11.21	67.8
	51.7	40.8	12.39	9.82	79.26	128.7	71.4	15.54	68.2
P. O. J. 234	.0	.0	18.06	15.85	87.76	219.8	-------	9.10	71.1
	25.8	.0	17.53	15.32	87.39	211.9	7.9	9.44	68.7
	47.5	34.6	16.11	13.33	82.74	179.1	40.7	11.17	67.7
Co. 281	.0	.0	16.78	14.38	85.70	197.1	-------	10.15	71.1
	25.0	.0	16.13	13.66	84.70	185.8	11.3	10.76	69.7
	47.5	34.6	14.73	11.67	79.23	152.8	44.3	13.09	68.0

The diseased and sound samples of P. O. J. 213 and P. O. J. 36–M were analyzed further, to determine whether the nonsugars were affected by borer and red rot injury. From the data of table 2 it is seen that the nonsugars were affected as greatly as was the carbohydrate fraction of the juice. No certain division between the changes produced by the borer and that by the fungus can be made from the data since no samples were available that were free from borer injury yet infected with red rot.

TABLE 2.—*Composition of nonsugars of crusher juice of sound and of injured*[1] *sugarcane, expressed as percentage of Brix solids*

Variety and condition of cane	Total nitrogen	Protein nitrogen	Nonprotein nitrogen	Gums	Ash	Potassium as K_2O	Calcium as CaO
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Wholly sound	0.165	0.074	0.091	0.133	2.093	1.075	0.059
Injured by borer	.192	.092	.100	.177	3.078	1.634	.069
Injured by borer and red rot	.317	.131	.186	.383	5.105	3.034	.092
P. O. J. 36–M:							
Wholly sound	.119	.068	.051	.177	2.251	1.019	.072
Injured by borer	.154	.079	.075	.196	2.672	1.397	.073
Injured by borer and red rot	.206	.100	.106	.246	3.489	1.780	.101

Variety and condition of cane	Magnesium as MgO	Aluminum as Al_2O_3	Iron as Fe_2O_3	Phosphate as P_2O_5	Sulphur as SO_3	Silica as SiO_2	Acidity[2]
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cc*
Wholly sound	0.110	0.028	0.020	0.332	0.271	0.110	1.35
Injured by borer	.110	.044	.045	.334	.350	.116	1.55
Injured by borer and red rot	.186	.174	.047	.494	.552	.157	2.05
P. O. J. 36–M:							
Wholly sound	.140	.092	.038	.392	.315	.113	1.50
Injured by borer	.128	.107	.013	.454	.247	.127	1.90
Injured by borer and red rot	.158	.087	.032	.509	.290	.156	2.00

[1] For extent of injury see second and third columns of table 1.
[2] Phenolphthalein titration, cubic centimeters of 0.1 N alkali required to neutralize 10 cc of juice.

The total nitrogen of P. O. J. 213 increased 16 percent and that of P. O. J. 36–M increased 29 percent as a result of borer injury. When accompanied by red rot, the total nitrogen of these samples increased 92 and 73 percent, respectively, above that found for sound cane. Although the protein-nitrogen content of the juice is materially increased as a result of the infestations, the proportion of the total nitrogen in the protein form appears to be somewhat decreased, with a corresponding increase in the nonprotein fraction. The protein nitrogen decreased from 57 percent of the total in the sound P. O. J. 36–M to 51 percent in the borer-injured sample and to 49 percent in the sample injured by both borer and red rot. The data on the 1931 samples of P. O. J. 213 are less conclusive, the protein nitrogen increasing from 45 percent of the total in sound cane to 48 percent in the bored sample, and decreasing to 41 percent in the bored and red rot sample. The 1932 data, however, for P. O. J. 213, show a more definite decrease in the proportion of protein nitrogen to total nitrogen in the juice from damaged cane as compared with that from sound cane.

The acidity of the juice of both varieties was increased by the borer and particularly by the combined action of borer and red rot. The amount of gums present in the juice was also much increased by the

borer and red rot, and, to a lesser extent, by borer damage alone. While borer damage alone increased the ash content of the P. O. J. 213 samples approximately 50 percent, borer damage combined with red rot increased the ash content 144 percent in the samples examined. Similar but somewhat less marked effects were noted in the case of P. O. J. 36–M, where the juice ash was increased 19 percent by borer injury alone and 55 percent by borer injury and red rot combined. The quantity of each inorganic component present in the juice as found in the ash was likewise increased by borer and red rot injury.

The analyses of the sirups produced from these juices are given in table 3.

TABLE 3.—*Composition of sirups of sound and of diseased* [1] *cane expressed as percentages of true solids*

Variety and condition of cane	True purity	Reducing sugars	Ash	Total nonsugars	Organic nonsugars	Nitrogen as N	Gums	Organic substances precipitated by alcohol
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Wholly sound	91.02	2.67	2.265	6.31	4.05	0.105	0.094	0.60
Injured by borer	89.73	2.70	3.163	7.57	4.41	.135	.147	.95
Injured by borer and red rot	82.63	5.78	5.364	11.60	6.24	.215	.331	2.33
P. O. J. 36–M:								
Wholly sound	89.88	3.66	2.156	6.46	4.30	.074	.092	.76
Injured by borer	86.06	6.68	2.776	7.26	4.48	.091	.114	.96
Injured by borer and red rot	82.13	9.13	3.645	8.74	5.09	.129	.211	1.17

	Silica as SiO_2	Sulphur as SO_3	Iron as Fe_2O_3	Calcium as CaO	Increase in CaO due to clarification	Color number [2]	Turbidity number [2]
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		
Wholly sound	0.061	0.334	0.0012	0.181	0.122	88	88
Injured by borer	.110	.416	.0042	.191	.122	187	386
Injured by borer and red rot	.121	.617	.0056	.437	.345	342	432
P. O. J. 36–M:							
Wholly sound	.078	.393	.0014	.194	.122	56	49
Injured by borer	.064	.438	.0021	.223	.150	103	77
Injured by borer and red rot	.087	.540	.0032	.344	.243	117	280

[1] For extent of injury, see second and third columns of table 1.
[2] Color and turbidity numbers are 100 times—log t. The larger numbers represent greater color and turbidity.

The data show that changes effected by borer and red rot on the juice purity, ash, gums, and nitrogen were carried through to the sirup. In addition, an increased amount of reducing sugars and of organic nonsugars was found in the sirups from injured cane; the color was much darker and the turbidity greater. The increased color and turbidity are especially objectionable because of their detrimental influence on the quality of the sugar. It is to be noted also that the increase in lime content due to clarification is greater in the case of sirup from the cane having red rot. This unusual increase in soluble lime salts cannot be explained solely on the basis of the higher acidity of the juice but may be due to the kind of acids present.

When the values of the various inorganic substances present in the juice are recalculated to percentage composition of the ash, as in table 4, it is seen that the composition of the ash is not greatly altered

as a result of the infection, even though the total quantity is increased. The principal change was an increase in the proportion of potassium in the ash and a decrease in most of the other constituents.

TABLE 4.—*Composition of the ash of crusher juice of sound and of diseased sugarcane*[1]

Variety and condition of cane	Potassium as K_2O	Calcium as CaO	Magnesium as MgO	Aluminum as Al_2O_3	Iron as Fe_2O_3	Phosphorus as P_2O_5	Sulphur as SO_3	Silica as SiO_2
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Wholly sound	51.36	2.82	5.26	1.34	0.96	15.86	12.95	5.26
Injured by borer	53.09	2.24	3.57	1.43	1.46	10.85	11.37	3.77
Injured by borer and red rot	50.43	1.80	3.64	3.41	.92	9.68	10.81	3.08
P. O. J. 36–M:								
Wholly sound	45.27	3.20	6.22	4.09	1.69	17.41	13.99	5.02
Injured by borer	52.28	2.73	4.79	4.00	.49	16.99	9.24	4.75
Injured by borer and red rot	51.02	2.89	4.53	2.49	.92	14.59	8.31	4.47

[1] For extent of injury see second and third columns of table 1.

TESTS IN 1932

The 1931 results were confirmed by the 1932 tests on sound and on diseased cane of the varieties P. O. J. 213 and Co. 281. The samples collected in 1932 were intended to represent maximum conditions of natural infection in the case of two varieties commercially grown in Louisiana that have different degrees of susceptibility to these injuries, P. O. J. 213 being highly susceptible to damage by both borer and red rot (*1*), and Co. 281 being quite resistant to red rot although somewhat susceptible to damage in the field by the borer. As indicated by the analyses given in table 5, the sample of diseased P. O. J. 213 has only 54 percent of the apparent sucrose and 68 percent of the solids content of the juice of comparable healthy cane, thus indicating a loss of over 117 pounds of 96° sugar per ton of cane. The data for Co. 281 show this variety to be less seriously changed in composition than P. O. J. 213, even when apparently 100-percent infected. The soluble solids and apparent sucrose decrease of 13 percent and 20 percent, respectively (table 5), indicate a loss of sugar, due to the diseased condition of over 45 pounds per ton of cane.

TABLE 5.—*Effect of borer and red rot injury on the sucrose content of crusher juice from two varieties of sugarcane, United States Sugar Plant Field Station, Houma, La., 1932*

Variety and condition of cane	Brix	Apparent sucrose	Apparent purity	Indicated 96° sugar per ton of cane		Cane for 1 ton of sugar
				Total	Loss due to injury	
P. O. J. 213:	*Degrees*	*Percent*	*Percent*	*Pounds*	*Pounds*	*Tons*
Sound	17.51	15.76	90.01	221.3	----------	9.04
Diseased	11.96	8.52	71.24	104.2	117.1	19.19
Co. 281:						
Sound	17.09	14.14	82.74	190.0	----------	10.53
Diseased	14.84	11.30	76.15	144.4	45.6	13.85

The nonsugar analyses (table 6), give further evidence that red rot and borer injury have a greater effect on P. O. J. 213 than on Co. 281. The total nitrogen content of the juice from the former increased

125 percent as a result of infection, and that of the latter increased 51 percent. In both varieties the relative proportion of protein and nonprotein nitrogen was changed by the infestation, the protein nitrogen being 67 percent of the total in the juice of sound P. O. J. 213 and 55 percent of the total in the juice of the diseased samples and 69 and 66 percent, respectively, in the sound and diseased Co. 281. The ash content of both varieties increased because of the injury, the increase being over 200 percent in P. O. J. 213 and 37 percent in Co. 281. Nearly all the mineral constituents of the juice appear to be correspondingly increased. Calculation of the ash constituents as percentages of the ash (table 7) confirmed the previous data in that the potassium content of the juice was increased somewhat more than the other components as a result of injury by borer and red rot.

TABLE 6.—*Composition of the nonsugars of crusher juice of sound and of diseased sugarcane, expressed as percentages of Brix solids*

Variety and condition of cane	Total nitrogen	Protein nitrogen	Nonprotein nitrogen	Ash	Potassium as K_2O	Sodium as Na_2O	Calcium as CaO
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sound	0.060	0.040	0.020	1.673	0.915	0.033	0.029
Diseased	.135	.074	.061	5.043	2.865	.071	.070
Co. 281:							
Sound	.070	.048	.022	3.191	1.896	.044	.107
Diseased	.106	.070	.036	4.376	2.686	.067	.106

Variety and condition of cane	Magnesium as MgO	Manganese as MnO	Phosphorus as P_2O_5	Sulphur as SO_3	Chloride as Cl	Silica as SiO_2	Acidity[1]
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cc*
Sound	0.064	0.0013	0.409	0.091	----------	0.088	1.22
Diseased	.121	.0029	.991	.290	----------	.112	1.74
Co. 281:							
Sound	.145	.0020	.441	.275	0.141	.081	2.32
Diseased	.138	.0019	.518	.353	.251	.097	2.42

[1] Electrometric titration to pH 7.0, cubic centimeters of 0.1 N alkali required to neutralize 10 cc of juice.

TABLE 7.—*Composition of the ash of crusher juice of sound and of diseased sugarcane*

Variety and condition of cane	Potassium as K_2O	Sodium as Na_2O	Calcium as CaO	Magnesium as MgO	Manganese as MnO	Phosphorus as P_2O_5	Sulphur as SO_3	Chlorine as Cl	Silica as SiO_2
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sound	54.69	1.97	1.73	3.83	0.077	24.45	5.44	--------	5.26
Diseased	56.81	1.41	1.39	2.40	.058	19.65	5.75	--------	2.22
Co. 281:									
Sound	59.42	1.38	3.35	4.54	.063	13.82	8.62	4.42	2.53
Diseased	61.38	1.53	2.42	3.15	.043	11.84	8.07	5.74	2.22

The analyses of the clarified juices (table 8) reaffirmed the purity drop in diseased cane, and, as in the previous tests, showed an increase in the proportion of reducing sugars, ash, and organic nonsugars and a poorer degree of clarity given by a standard sugar-house clarification. As in the case of the raw juice, the changes were greater in the clarified juice of P. O. J. 213 than in that of Co. 281.

TABLE 8.—*Composition of lime-clarified juices from sound and from diseased sugarcane, expressed as percentages of true solids*

Variety and condition of cane	True purity	Reducing sugars	Ash	Total non-sugars	Organic non-sugars	Turbidity number [1]
P. O. J. 213:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Sound	93.87	1.87	1.70	4.26	2.56	43
Diseased	80.40	9.06	4.85	10.54	5.69	59
Co. 281:						
Sound	90.10	3.77	3.00	6.13	3.00	28
Diseased	85.00	5.92	4.41	9.18	4.41	40

[1] The turbidity numbers are 100 times the reciprocal of the Kopke value. The larger numbers represent greater turbidity.

SUMMARY

The composition of juice from sugarcane damaged by the sugarcane borer alone and from cane damaged by both the borer and red rot is contrasted with that of juice from sound cane. The comparisons show that the value of the cane is materially reduced by the borer alone and to a greater extent by the combination of borer and red rot injuries. In both cases the following changes occur as a result of the injuries.

There is a decrease in juice extraction and in the percentage of solids and sucrose in the juice obtained, and the apparent and the true purity are correspondingly lowered.

The percentages of reducing sugars, ash, gums, alcohol-precipitable nonsugars, and total organic nonsugars are significantly increased.

Both protein and nonprotein nitrogen compounds are increased, especially the latter, resulting in a greater increase in the nitrogen content of the sirups.

In the ash, potassium is increased somewhat more than the other elements, although all the mineral constituents of the juice are increased. When calculated to percentage of ash, the ratios between the different components are but slightly altered.

The color and the turbidity of the clarified juices and sirups are decidedly increased.

A comparison of four varieties commercially grown in Louisiana indicates that, in general, the changes in the chemical composition of the juice are greater in the case of the highly susceptible variety P. O. J. 213 than in the other varieties tested.

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INHERITANCE OF SOME MAJOR COLOR TYPES IN BEETS[1]

By WESLEY KELLER[2]

Formerly *agent, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The occurrence and distribution of red and yellow pigments in beets (*Beta vulgaris* L.), as has generally been recognized, give rise to a large number of heritable color types. But even with such possibilities for genetic study, inheritance in beets has received comparatively little attention, although plant-breeding efforts specially concerned with the sugar content of beets had been in progress for almost a century before the rediscovery of Mendel's principles.

This lack of genetic advancement may in part be attributed to certain characteristics of the beet. Owing to its strong tendency toward self-sterility, populations large enough for study are not readily obtained by inbreeding.[3] When a cross is desired there is usually some selfing. Because of the small size of the beet flower, extensive emasculation is impracticable. Another important reason for the lack of genetic advancement with beets is that many of the important beet characters are readily modified by changes in environmental conditions. In this respect color is no exception. In these studies it was found that the development of both red and yellow root pigments is greatly influenced by the physiological condition of the plant, and under some conditions the effect upon the yellow pigment has been such as to cause serious difficulty in classifying certain genetic phenotypes. A more comprehensive knowledge of the physiological conditions which cause these variations and the discovery of clear-cut genetic characters that can be identified under all conditions will constitute important advances in breeding work with beets.

Kajanus[4] made numerous crosses, which were carried several generations, with beets of the mangel-wurzel type, chiefly of the Eckendorfer and Barres varieties. His data did not lead to a satisfactory genetic analysis because of a persistent and high percentage of contamination, perhaps the result of his use of cloth tents as plant isolators. He was able to present a factorial arrangement, partly hypothetical, by considering large discrepancies to be the effect of contamination. This arrangement assumed a yellow root to be the effect of a dominant factor for yellow, designated *G* (from Gelb), and a red root to be the effect of a dominant factor for red, *R*, in

[1] Received for publication Oct. 2, 1935; issued February 1936. Cooperative studies by the U. S. Department of Agriculture and the Utah Agricultural Experiment Station.

[2] The writer acknowledges many helpful suggestions in the preparation of the manuscript from F. V. Owen, geneticist, and other members of the Division of Sugar Plant Investigations, as well as from George Stewart, senior forest ecologist, Intermountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture.

[3] Fully self-fertile strains are now well known to investigators in the Division of Sugar Plant Investigations, but they were not utilized in the study reported in this paper.

[4] KAJANUS, B. ÜBER DIE FARBENVARIATION DER BETA-RÜBEN. Ztschr. Pflanzenzücht. 5:[357]-372. 1917.

combination with the factor for yellow. A beet of the phenotype *Rg* had a white root, but true whites were of the genotype *rrgg*. He considered these two factors to be independently inherited.

Lindhard and Iversen [5] repeated the work of Kajanus and reached similar conclusions. They believed that some of their data suggested a loose linkage between the *Rr* and *Gg* factor pairs, but this was not consistent with other related data. Rasmuson [6] reported the result of a cross of red beet with a white beet; the color under consideration was presumably confined to the epidermis of the root. Rasmuson thought his data conformed to Kajanus' scheme. Vilmorin [7] studied numerous progenies of "accidental" crosses and reported that the ratios obtained were in general agreement with the findings of Lindhard and Iversen.

The present paper deals with nine color types, obtained from crosses between sugar beets and red beets, and the genetic factors responsible for their inheritance. Crosses were obtained by exposing the mother plant to pollen from the selected male parent. This has been effectively accomplished in two ways: (1) By distance isolation and (2) by the use of paper bags. According to the first method plants were usually grown in nail kegs, and shortly before the flowers opened the paired parent plants were moved to some place at least 200 to 300 yards from the nearest flowering beets. When paper bags were used small branches were bagged shortly before the earliest flowers opened, and a few days later, when about half the flowers had opened and shed their pollen, each bag with its contents was exchanged for a similar bag on the reciprocal parent.

All of the crosses reported in the section on factors *R* and *Y* were made by distance isolation, while most of the crosses in the section on the factors R^t and Y^r were made under paper bags. A small amount of contamination may have occurred with either method, but this should not be more serious than in the case of maize or any other cross-pollinated plant.

Both distance isolation and the use of paper bags provide efficient methods of obtaining crosses, but offer no protection against a small percentage of selfing which commonly occurs in beets. It is, of course, important that the breeder be able to recognize those individuals resulting from selfing, and where the parental phenotypes are otherwise similar a color character is frequently extremely useful in distinguishing parentage. If, however, the phenotype of the female parent is expected in the progeny, specific individuals resulting from selfing could not be detected, but the effect of selfing on the population might be recognized by an excessive number of plants in the particular class whose phenotype is also that of the female parent. In the work reported here, the effect of self-fertility has not been entirely avoided, but through the use of simple methods it is believed that pollination has been controlled well enough to permit effective interpretation of the genetic behavior.

[5] LINDHARD, E., and IVERSEN, K. VERERBUNG VON ROTEN UND GELBEN FARBENMERKMALEN BEI BETA-RÜBEN. Ztschr. Pflanzenzücht. 7: 1–18. 1919.

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[7] VILMORIN, J. L. DE. L'HÉRÉDITÉ CHEZ LA BETTERAVE CULTIVÉE. 153 pp., illus. Paris. 1923.

DESCRIPTION OF COLOR TYPES

At least three color types (red, yellow, and white root) were noted in the literature reviewed, but because of a lack of adequate description of the plants studied it is impossible to establish an accurate relationship between these color types and those which form the basis of the present paper. This paper includes nine color types, which for the purpose of description are divided into two groups: (1) Those containing red pigment and (2) those lacking red pigment.

TYPES CONTAINING RED PIGMENT

(I) *Red-hypocotyl white root* (*Ry*).—The hypocotyl of the seedlings of this type is rose or pale red. The color shows also at the bases of stems and petioles, the intensity depending upon environmental conditions and the stage and rapidity of growth. Genetic modifying factors also may be responsible for different shades and intensities of pigments observed or there may be an allelomorphic series of color factors, each factor having a slightly different expression. Sometimes the pigment is not readily seen in a large vegetative beet, and this may account for some of the confusion in the literature. By cutting down to the central bud, however, as has been pointed out by Nuckols,[8] the pigment can be detected easily. This color character is a specific marker that is expressed at all times, although under certain conditions during the formation of the seedstalk only traces of the pigment remain. Sometimes the pigment also gives part of the skin on the shoulders of the root a tinge of red.

(II) *Red beet* (*RY*).—In the cotyledon or seedling stage, the hypocotyl of beets belonging to the red beet type is of an intensely dark or purplish-red color. In the growing and in the mature plant the petiole color may range from a dark purplish red to an orange-red, or it may be red in the middle and orange at the margins. In the leaves the pigment is most concentrated in the midrib and principal veins,[9] but under certain favorable conditions the entire leaf may be dark red. The epidermis of the root is dark red, and the flesh of the root is predominantly red.

(III) *Pale red beet* (*RY*±?).—The color in this type extends into every part of the plant characterized by red color in type II, but with greatly reduced intensity.

(IV) *Striped red beet* (RY^{s}).—The root and flesh color is intermediate in intensity between types II and III. The color of the petioles is a dark or purplish red similar to that of type II, but it is confined chiefly to stripes. On the upper surface of the petiole the color is usually confined to one broad stripe, but on the underside there are several narrow stripes of red. Orange has never been found on petioles of this type. Only rarely does the red pigment extend into the leaf veins. When it does so, it is extremely dilute.

(V) *Green top red root* (RY^{r}).—The roots of this type vary in color from medium to bright red and cannot be distinguished from the roots of type IV, while in the tops the pigment is generally as restricted as in those of type I. In mature plants there is an abrupt change from the presence of red to its absence or only a trace, at the intersection of crown and petioles. Only one beet of this description was found in 1932, and it became the progenitor of all the beets with green tops and red roots discussed in this paper. The type has probably been observed by earlier workers, but, so far as the writer is aware, there has been no previous description.

(VI) *Red top white root* ($R^{t}y$).—Roots of this type are similar to those of type I. They generally show a trace of rose on the shoulders, but under most conditions they are white. The tops are striped with red and cannot be distinguished from those of type IV. In mature plants there is an abrupt change from the presence of intense red to its absence or to a rose tinge, at the intersection of crown and petioles.

TYPES LACKING RED PIGMENT

(VII) *Yellow hypocotyl white root* (*ry*).—In the cotyledon and seedling stages, beets of this type have a whitish-green hypocotyl. Upon exposure to sunlight,

[8] NUCKOLS, S. B. SEEDLING COLOR AND YIELD OF SUGAR BEETS. Jour. Amer. Soc. Agron. 23: 740-743. 1931.

[9] The concentration of pigment in the veins is particularly interesting in comparison with a color type which F. R. Immer obtained from Germany. In the latter type the expression is just opposite, i. e., the pigment in the leaf blades is most concentrated away from the veins.

considerable yellow pigment may develop, however. The bases of the leaves sometimes show a trace of yellow, depending upon environmental conditions, stage of growth, etc. This type corresponds to type I, except that here the pigment is yellow instead of red, and therefore is usually not so conspicuous.

(VIII) *Green top yellow root* (*rYr*).—The petioles and leaves of this type are similar in color to those of type VII, but the epidermis of the root and sometimes part of the flesh and vascular rings are a lemon yellow.

(IX) *Yellow beet* (*rY*).—The yellow pigment in this type is distributed throughout the plant as is the red pigment in type II. The petioles are usually a distinct yellow. The color may be confined to the petioles of young leaves or it may extend into the veins of every green leaf on the plant. When strongly displayed this petiole coloration is a clear golden yellow. The epidermis of the root is lemon yellow except on the shoulders, where in some plants it blends into orange. When the orange is absent the lemon-yellow root color is similar to that of type VIII but is usually more intense. Under some conditions it is impossible to distinguish the roots of this type from those of type VIII.

METHOD OF DISTINGUISHING THE YELLOWS OF TYPES VII, VIII, AND IX

In the greenhouse, high temperature and possibly other conditions favorable to rapid growth are so unfavorable for the expression of yellow color as to make it impossible to identify the different types that lack red pigment. Observations made by F. V. Owen have shown that there is a remarkable development of all pigments in the root if young plants grown under greenhouse conditions are pulled and laid in sunlight on damp soil for a few days. Following this exposure to sunlight the presence or absence of factors for yellow pigment can usually be determined. Before the plants were subjected to this treatment, it was particularly difficult to distinguish between the white roots of type VII and the yellow roots of type VIII. Under field conditions, the lemon-yellow color of type VIII is clear-cut and can be easily identified.

THE FACTORS *R* AND *Y* IN THE RED GARDEN BEET

MATERIAL

A beet with a red hypocotyl and a white root, of genetic constitution *Ryry*, was crossed with a red beet of genetic constitution *RYRY*. The cross was made in 1930 by growing the two parent beets side by side in a city garden, where, so far as could be ascertained, they were isolated from other flowering beets by a distance of at least 300 yards. The red-hypocotyl white-root parent (tpye I) was selected from the P–19 strain [10] (a curly-top resistant strain of sugar beet), and the red beet parent (type II) was a table beet of the Detroit Dark Red variety, a commercial variety characterized by a maximum development of intense red pigment. From this cross, approximately 30 plants were grown from seed harvested from the red-hypocotyl white-root parent (*Ryry*). These F_1 plants were forced to seed under lights in the greenhouse and allowed to cross with each other at random. In 1931 a large population of both the F_1 and F_2 was grown in the field.

If crossing had been complete, all offspring in the F_1 planting would be expected to resemble the dominant red beet parent. This did not prove to be the case, however, and some self-fertilization must have taken place since a few plants with white roots were represented in the progeny. Furthermore, these white-root selfed plants were of two types, the red hypocotyl (*Ry*) and the yellow hypocotyl (*ry*),

[10] Obtained by the Utah Agricultural Experiment Station from Katherine Esau, formerly with the Spreckels Sugar Co., and now a member of the staff of the University of California, Davis, Calif.

which demonstrated that the white-root parent (P–19) was heterozygous for hypocotyl color, and hence of genetic constitution *Ryry*.

Since the composite F_2 obtained could not yield a satisfactory factorial analysis (because of the mixed population of genotypes *RYRy* and *RYry* in the F_1) several F_1 plants were backcrossed to the double recessive yellow-hypocotyl white-root type (*ryry*). These crosses, together with further evidence from additional combinations, provided ample proof of the presence of two factors, namely, *Y* for the extension of pigment and *R* for the conversion of yellow to red pigment, which is in line with the results of earlier investigators.

F_1 BACKCROSSES

The first generation genotypes *RYry* and *RYRy* were both backcrossed to the double recessive *ryry*. Backcrosses of the first group (*RYry*) to the yellow-hypocotyl white-root type (*ryry*) gave four phenotypes, *RY*, *Ry*, *rY*, *ry*, with a close linkage between *R* and *Y*. The data suggest approximately 7.5 percent crossing over. These backcrosses are summarized in table 1, crosses 1 and 2. In each of these crosses several progenies were combined to simplify the presentation. Each progeny had previously been found to be in satisfactory agreement with expectation.[11] The results of these backcrosses are taken as confirming the genetic interpretation given, large deviations from the expected numbers usually being attributed to selfing. Throughout the paper this effect of selfing will be seen operative in accentuating the percentage crossing over or in reducing the value of *P* when the χ^2 test was used. The interpretations as given have been based upon reasonably consistent behavior of many progenies and are believed to warrant the factorial explanations given.

TABLE 1.—*Linkage between the Rr and Yy factor pairs*

Cross no.	Cross	Total number of plants	Number of plants of indicated phenotype				Percentage crossover ± P. E.
			Red beet (type II or III) [1] *RY*	Red hypocotyl white root (type I) *Ry*	Yellow beet (type IX) *rY*	Yellow hypocotyl white root (type VII) *ry*	
1	*RYry*×*ryry*	484	242	19	18	205	7.6±0.81
2	Reciprocal	443	202	20	14	207	7.7± .85
3	*RYry*×*ryry*	111	58	3	2	48	4.5±1.33
4	Reciprocal	654	282	14	14	344	4.3± .53
5	*RYry*×*ryry*	102	51	2	3	46	4.9±1.44
6	Reciprocal	353	172	12	6	163	5.1± .79
7	*RYry*×*RYry*	67	48	4	1	14	6.3±2.08
8	Reciprocal	311	216	10	12	73	7.1±1.03
9	*RrYY*×*ryry*	63	4	27	29	3	11.1±2.68
10	Reciprocal	41	2	20	12	7	22.0±4.36
11	*RrYY*×*ryry*	101	4	50	45	2	5.9±1.58
12	Reciprocal	85	2	30	36	17	22.4±3.05

[1] Type III in crosses 9 to 12.

[11] Probable errors were calculated by the aid of tables prepared by Immer.[12] In some cases the χ^2 test was most convenient.

[12] IMMER, F. R. FORMULAE AND TABLES FOR CALCULATING LINKAGE INTENSITIES. Genetics 15:81–98, illus. 1930.

In backcrosses of the second group ($RYRy \times ryry$), two phenotypes (RY and Ry) were expected in about equal numbers. The actual ratio was 341 to 284, with P (from χ^2) falling between 0.02 and 0.05; the excess of red plants, however, suggests that there was some self-fertility in the female parent. The reciprocal gave 202 RY to 168 Ry, with P falling between 0.05 and 0.10. This progeny also contained 24 plants of the yellow-hypocotyl white-root type ($ryry$), but these were without doubt the result of selfing. The figures presented above for the backcrosses of $RYRy$ to $ryry$ and the reciprocals are also the sums of several progenies. For the individual progenies, the lowest value for P was between 0.05 and 0.10 and the highest was between 0.8 and 0.9.

F_2 BACKCROSS AND F_3 POPULATIONS

Except that the linkage seems to be slightly closer, the F_2 backcross and F_3 populations are similar to the F_1 backcross populations (crosses 1 and 2) and help to corroborate the genetic interpretation. These data are presented in table 1, crosses 3 to 8, inclusive. In at least one population (cross 4) the closeness of the apparent linkage was accentuated by self-fertility in the yellow-hypocotyl white-root parent ($ryry$).

Additional interest centered in these progenies because in each group one of the red parents showed distinct orange margins on the petioles, while the other red parent had intense red petioles with no evidence of orange. A genetic interpretation for orange margins, as contrasted with lack of orange (both are of color type II), was not obtained because none of the segregates showed a distinct orange, although a few showed a trace. Since the plants were grown in a dense stand, it is possible that orange color requires a particular environment, failing to develop except when the plants are exposed to intense sunshine and there is adequate spacing between plants.

The yellow beet rY (type IX) was shown to be produced as a simple dominant to the double recessive in a backcross of a heterozygous yellow beet ($rYry$) to the yellow-hypocotyl white-root type ($ryry$). The expected ratio was 1 yellow beet to 1 yellow hypocotyl white root, and the observed numbers were 231 to 253, with P falling at 0.3.

THE PALE RED BEET

The F_1 population grown in 1931 included one unusual plant which was a pale-colored red beet. This plant proved to be self-sterile, but when it was backcrossed to the yellow-hypocotyl white-root type ($ryry$) a total progeny of 290 individuals was obtained. The data are presented in table 1, crosses 9 to 12, inclusive.

Although the linkage could not be accurately measured, chiefly because of self-fertility in the recessive female parents, the ratios (though not the color) were satisfactorily explained by assuming the pale red beet (type III) to be of the genotype $RyrY$. This repulsion arrangement is particularly significant because all of the other doubly heterozygous F_1 plants were of the coupling type ($RYry$). However, half of the gametes produced by the sugar beet parent ($Ryry$) were of the Ry type, and consequently both the repulsion arrangement and the dilute color of the pale red beet could be obtained by the introduction of a gamete of the rY^d type, Y^d being a dilute allelomorph of Y.

There is not sufficient evidence available at present, however, to propose such a factor; consequently, the well-known factor *Y* will be used, it being understood that the correct genotype of the pale red beet has not been determined.

The pale red beet was most likely the result of contamination, but there are other explanations based upon genetic behavior which cannot be entirely disregarded.

RECOVERY OF THE PARENTAL TYPES

The parental types *RY* (red beet) and *ry* (yellow hypocotyl white root) have been recovered by crossing the heterozygous recombination types, red hypocotyl white root (*Ryry*) × yellow beet (*rYry*). Such a cross constitutes a critical test of the dependability of the genetic interpretation used to explain the color relations observed. The expected ratio 1:1:1:1 is approached by the observed ratios, as shown in table 2. The population obtained from the red-hypocotyl white-root plant as female parent suggests that considerable self-fertilization occurred.

TABLE 2.—*A heterozygous yellow beet (rYry) × a heterozygous red hypocotyl white root (Ryry), and the reciprocal (Ryry × rYry)*

Cross	Total number of plants	Number of plants of indicated phenotype				*P* from χ^2 test
		Red beet (type II) *RY*	Red hypocotyl white root (type I) *Ry*	Yellow beet (type IX) *rY*	Yellow hypocotyl white root (type VII) *ry*	
rYry × *Ryry*	[1] 200	53	38	50	59	0.2
Reciprocal	[2] 339	65	[3] 117	62	95	<.01

[1] Calculated number of plants of each type, 50.
[2] Calculated number of plants of each type, 84.75.
[3] The excess in this class shows distinct evidence of self-fertility.

THE FACTORS R^1 FOR RED TOP AND Y^r FOR ROOT COLOR [13]

MATERIAL

The remaining portion of this paper deals with color types obtained from a red beet (origin unknown), the genetic interpretation of which is entirely different from that previously given. The red beet was not observed during its vegetative stage, and consequently its characteristics are known only from the red beet segregates in its progeny. These plants were not so intensely pigmented as the Detroit Dark Red variety, and the petiole pigmentation was confined to a broad stripe which usually did not extend into the midrib or veins of the leaf. These plants were of the type designated as striped red beet (type IV).

The white-root beet originally crossed with the striped red beet was a selection from a Russian sugar beet strain known as F29–01. These parent plants were isolated in 1930 by George Stewart, formerly agronomist at the Utah Agricultural Experiment Station.[14]

[13] Most of the progenies reported in this section were grown by F. V. Owen.
[14] Dr. Stewart left the Utah station in the fall of 1930, turning this material over to the writer, who had been working under his supervision.

The cross of striped red beet × red hypocotyl white root yielded only about a dozen seeds, which were all from the striped red beet parent. A few F_1 plants were grown in the greenhouse during the winter of 1930–31, and the stecklings obtained were isolated in one group in the field in 1931. The remaining seeds produced a few F_1 plants which were grown in the field the same year. In 1932, when an F_2 population was grown to maturity, a most interesting segregation occurred. Three types not previously encountered were found, each requiring a modification of the RY explanation previously employed. These types are (1) red top white root (type VI), assumed to be due to a single factor R^t; (2) green top yellow root (type VIII), assumed to be due to a single factor Y^r; and (3) green top red root (type V), assumed to be due to a combination of R (factor for red hypocotyl) plus Y^r (factor for root color). It is interesting that the striped red beet should have resulted from the combination of R^t (red top) and Y^r (root color).

F_1 BACKCROSSES ($R^tY^rry \times ryry$)

From the original cross of striped red beet × red hypocotyl white root, one F_1 plant of genetic constitution R^tY^rry was clonally propagated, two pieces of which matured seeds in backcrosses to beets of the yellow-hypocotyl white-root type ($ryry$). When the progenies from these crosses were grown, segregates included plants of the green-top yellow-root type (rY^r) and the red-top white-root type (R^try). The data from these crosses are presented in table 3. They show a close linkage between R^t and Y^r, with approximately 7.5 percent crossing over. The linkage is similar to that between Rr and Yy, which has already been discussed.

TABLE 3.—*Striped red beet* (R^tY^rry) × *the double recessive* ($ryry$), *and the reciprocals* ($ryry \times R^tY^rry$)

Cross	Total number of plants	Number of plants of indicated phenotype				Percentage crossover ±P. E.
		Striped red beet (type IV) R^tY^r	Red top white root (type VI) R^try	Green top yellow root (type VIII) rY^r[1]	Yellow hypocotyl white root (type VII) $ryry$	
$ryry \times R^tY^rry$	230	97	14	5	114	8.4±1.24
Reciprocal	301	145	7	12	137	6.3± .94
$ryry \times R^tY^rry$	268	89	9	8	[2] 162	6.3±1.00
Reciprocal	265	133	12	11	109	8.7±1.17

[1] The factor Y^r could possibly be synonymous with Kajanus' G.
[2] The excess number of plants suggests that the female parent was rather self-fertile.

CROSSES WITH THE GREEN-TOP YELLOW-ROOT TYPE (rY^rry)

The genetic constitution of the green-top yellow-root type (rY^r) has been demonstrated in the progeny of crosses between it and four other color types. The data are presented in table 4. The various crosses are as follows:

(1) *Green top yellow root* (rY^rry) × *yellow hypocotyl white root* ($ryry$).—The expected ratio in this cross is 1 green top yellow root (rY^r) to 1 yellow hypocotyl

white root (*ry*), which is in close agreement with the observed ratio, *P* falling between 0.8 and 0.9 (table 4, cross 1).

(2) *Green top yellow root* (*rY^r ry*) × *red top white root* (*R^t yry*).—In this cross the recombination types from the F_1 backcross populations are reunited and the parental types are recovered (table 4, crosses 2 and 3). The first population contains an excess number of plants of the green-top yellow-root type, suggesting some self-fertility in the female parent.

TABLE 4.—*Crosses with the green-top yellow-root type* (*rY^r ry*)

Cross no.	Cross	Total number of plants	Number of plants of indicated phenotype							*P* from χ^2 test
			Striped red beet (type IV) *R^t Y^r*	Red top white root (type VI) *R^t y*	Red hypocotyl white root (type I) *Ry*	Green top red root (type V) *RY^r*	Yellow beet (type IX) *rY*	Green top yellow root (type VIII) *rY^r*	Yellow hypocotyl white root (type VII) *ry*	
1	*rY^r ry* × *ryry*	[1] 92						47	45	0.80–0.90
2	*rY^r ry* × *R^t yry*	[2] 54	14	7				21	12	.02– .05
3	Reciprocal	[3] 84	17	21				24	22	.70– .80
4	*rY^r ry* × *rY ry*	[4] 33					15	8	10	.70– .80
5	Reciprocal	[5] 76					32	24	20	.30– .50
6	*Ryry* × *rY^r ry*	[6] 154			38	36		45	35	.50– .70

[1] Calculated number of plants in each of the 2 types, 46.
[2] Calculated number of plants in each of the 4 types, 13.5.
[3] Calculated number of plants in each of the 4 types, 21.
[4] Calculated number of plants in the 3 types: 16.5, 8.25, and 8.25, respectively.
[5] Calculated number of plants in the 3 types: 38, 19, and 19, respectively.
[6] Calculated number of plants in each of the 4 types, 38.50.

(3) *Green top yellow root* (*rY^r ry*) × *yellow beet* (*rY ry*).—The expected ratio in this cross is 2 yellow beet (*rY*) to 1 green top yellow root (*rY^r*) to 1 yellow hypocotyl white root (*ry*), and the expected ratios are in good agreement with those observed (table 4, crosses 4 and 5).

(4) *Red hypocotyl white root* (*Ryry*) × *green top yellow root* (*rY^r ry*).—The progeny from this cross should segregate in the ratio of 1 red hypocotyl white root (*Ry*) to 1 green top yellow root (*rY^r*) to 1 green top red root (*RY^r*) to 1 yellow hypocotyl white root (*ry*). The actual segregation observed agrees very well with the theoretical ratio, *P* being between 0.5 and 0.7 (table 4, cross 6).

The data (table 4) apparently warrant the conclusion that the green-top yellow-root type (*rY^r*) is due to a single factor *Y^r*, which is dominant over the yellow-hypocotyl white-root type (*ryry*).

CROSSES WITH THE RED-TOP WHITE-ROOT TYPE (*R^t yry*)

The red-top character was found to be dependent upon a single factor (*R^t*). In backcrosses to the double recessive (*ryry*) a ratio of 1 red top white root (*R^t y*) to 1 yellow hypocotyl white root (*ry*) is expected. One progeny gave a ratio of 91 to 86, with *P* at 0.7; another gave 24 to 26, with *P* between 0.7 and 0.8; and a third gave 59 to 23, but the reciprocal of this cross (*ryry* × *R^t yry*) gave 15 to 10, with *P* at 0.3. The ratio of 59 to 23 was probably due to self-fertility, since the mother plant in the last cross was found to be rather self-fertile; upon selfing, it produced a progeny of 42 individuals in the ratio of 2 *R^t y* to 1 *ry*. The theoretical ratio for a single factor is 3 to 1, but the total number was small and the agreement proved to be satisfactory, *P* falling between 0.2 and 0.3.

Another cross, that of red top white root (R^1y) × green top yellow root (rY^r), and the reciprocal also support the single factor (R^1) concept. This cross has already been discussed and the data presented in table 4, crosses 2 and 3.

CROSSES WITH THE GREEN-TOP RED-ROOT TYPE ($RyrY^r$)

Two factors, R (red hypocotyl) and Y^r (yellow root) in combination, produce the green-top red-root type (RY^r). This was demonstrated in the cross of red hypocotyl white root (Ry) × green top yellow root (rY^r) which has already been discussed (table 4, cross 6). Three additional crosses are presented in table 5 in further support of this arrangement. A single plant of the green-top red-root type (RY^r) was used as the female parent in all three crosses. This plant was found to be of the genotype $RyrY^r$. The linkage between Rr and Y^ry appears to be similar to that between Rr and Yy, and consequently the calculated populations are based on a gametic ratio of 1 to 12. This linkage relationship also suggests that the factors for red and yellow, respectively, form two multiple allelomorphic series. Crosses with the green-top red-root type were made as follows:

(1) *Green top red root* ($RyrY^r$ × *yellow hypocotyl white root* ($ryry$).—The data from this cross are presented in table 5, cross 1. These data constitute conclusive evidence of close linkage in the repulsion phase. Three other similar crosses were studied, but in the progeny of each some striped red beets appeared. There is good reason to believe that these latter crosses were contaminated, probably because of a mistake in harvesting or in labeling the paper bags.

TABLE 5.—*Crosses with the green-top red-root type* ($RyrY^r$), *with calculated populations based on a gametic ratio of 1 to 12*

Cross no.	Cross	Total number of plants	Number of plants in indicated phenotype														Percentage cross-over ± P.E.	*P* from x^2 test
			Red beet (type II) RY^r		Striped red beet (type IV) R^1Y^r		Red top white root (type VI) R^1y		Red hypocotyl white root (type I) Ry		Green top red root (type V) RY^r		Green top yellow root (type VII) rY^r		Yellow hypocotyl white root (type VIII) ry			
			Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated		
1	$RyrY^r \times ryry$	682							315		23		307		37		8.8±0.73	
2	$RyrY^r \times R^1yry$	88			16	22	28	22	18	20.31	5	1.69	20	20.31	1	1.69		0.02–0.05
3	$RyrY^r \times rYry$	97	28	24.25					28	22.38	1	1.87	[1] 36	46.63	[2] 4	1.87		.05–.10

[1] Plants of the yellow beet type (rY) are included because of difficulties in classification, as explained in the text.
[2] May include some of type rY^r.

(2) *Green top red root* ($RyrY^r$) × *red top white root* (R^1yry).—The progeny from this cross segregated into six phenotypes, with P falling between 0.02 and 0.05, but by assuming a slight amount of self-fertilization a satisfactory fit is obtained (table 5, cross 2).

(3) *Green top red root* ($RyrY^r$) × *yellow beet* ($rYry$).—The data obtained from this cross are presented in table 5, cross 3. The agreement between the observed and expected populations is satisfactory, P falling between 0.05 and 0.1. This

population was harvested before a means of intensifying the pigment by exposure to sunlight was known; consequently, the two yellow classes are not separated. For the same reason, it is quite possible that some of the plants in the *ry* class should be in the yellow class, which would tend to improve the agreement between the observed and expected populations.

DISCUSSION

This paper reports a study of the inheritance of the major factors for red and yellow pigment in beets. The nine color types obtained from the progeny of two crosses are shown in table 6.

TABLE 6.—*Color types obtained from the progeny of two crosses*

Type no.	Type name	Phenotype	Type no.	Type name	Phenotype
I	Red hypocotyl white root	$R^t y$	VI	Red top white root	$R^t y$
II	Red beet	RY	VII	Yellow hypocotyl white root	ry
III	Pale red beet	[1] RY^r	VIII	Green top yellow root	rY^r
IV	Striped red beet	$R^t Y^r$	IX	Yellow beet	rY
V	Green top red root	RY^r			

[1] The writer was unable to determine the nature of the "additional influence" necessary to explain the light color of this type in contrast to the dark-red color of type II.

The investigations reported herein included a study of the progeny of a sugar beet with a red hypocotyl and a white root × Detroit Dark Red (an intensely red commercial variety of table beet). In this cross a close linkage with approximately 7.5 percent crossing over is noted between a factor for red hypocotyl (*R*) and a factor for yellow (*Y*). These two factors in combination (*R* plus *Y*) give rise to a red beet of the Detroit Dark Red type, and the absence of both *R* and *Y* gives the double recessive yellow-hypocotyl white-root type (*r* plus *y*).

This paper deals also with the progeny of a striped red beet (origin unknown) × a red-hypocotyl sugar beet having a white root. Segregation in the F_2 of this cross gave several plants with red tops and white roots, others with green tops and yellow roots, and one plant with a green top and a red root. Most of the data were satisfactorily explained by assuming the red-top white-root type to be due to a single factor (R^t), the green-top yellow-root type to be due to a single factor (Y^r), the striped red beet to be due to a combination of R^t and Y^r, and the green-top red-root type to be due to a combination of *R* and Y^r.

The linkage relation between R^t and Y^r is similar to that between *R* and *Y*, and it is thought that the factors for red pigment, *R* and R^t (plus the recessive *r*), form one allelomorphic series and the factors for yellow pigment, *Y*, Y^r, and *y* another series. Additional proof for this relationship is desirable, however, since data are not available on the linkage relationship between R^t and *Y*.

It is of special interest to note that *R* (factor for red hypocotyl), which is necessary for the expression of red color in the Detroit Dark Red variety, is widely distributed in commercial sugar-beet varieties. Therefore, the factor *Y* is the chief consideration in explaining the genetic constitution of the Detroit Dark Red variety, *Y* producing abundant yellow pigment and *R* changing the color to red. In con-

trast to this arrangement, the striped red beet is a combination of a factor (Y^r) for pigment in the root plus a factor (R^t) which produces red in the tops.

The factor R^t is dominant to R and increases pigment development in the top. Likewise, Y is considered dominant to Y^r and increases the abundance of pigment in the petioles and leaves, as well as causing pigment development in the root.

SUMMARY

The inheritance of color in nine types of beets obtained from crosses between sugar beets and red beets is reported.

One cross, between a red-hypocotyl white-root sugar beet (*Ryry*) × Detroit Dark Red table variety (*RYRY*), yielded four types: *RY* (red beet); *Ry* (red hypocotyl white root); *rY* (yellow beet); and *ry* (yellow hypocotyl white root). There is conclusive evidence of a close linkage between *Rr* and *Yy*, with approximately 7.5 percent crossing over. A pale red beet of similar genetic constitution was observed, but its complete genetic make-up for color was not learned.

A second cross between a striped red beet of unknown origin ($R^tY^rR^tY^r$)× a red-hypocotyl white-root sugar beet (*Ryry*) yielded five types: R^tY^r (striped red beet); R^ty (red top white root); rY^r (green top yellow root); RY^r (green top red root); and *ry* (yellow hypocotyl white root). The root color was noticeably less intense whenever Y^r replaced Y in the various genotypes. There is a close linkage, with approximately 7.5 percent crossing over, between R^t and Y^r and a similar linkage between R and Y^r.

It is believed that the R factors (R, R^t, and r) constitute one multiple allelomorphic series and the Y factors (Y, Y^r, and y) another series.

THE RELATION OF PAUSES TO RATE OF EGG PRODUCTION [1]

By I. MICHAEL LERNER, *research assistant in Poultry Husbandry*, and LEWIS W. TAYLOR, *head, Division of Poultry Husbandry, California Agricultural Experiment Station*

INTRODUCTION

Winter pause is an important character in the limitation of winter egg production during the pullet year. A great deal of the variability in winter production can be accounted for by pauses, differential winter rate, and sexual-maturity factors. The nature of winter pauses has been investigated by Hays (*1*, *2*, *3*)[2] and Hays and Sanborn (*4*, *5*, *6*), and both hereditary and environmental causes have been recognized (*2*). The present study was made to determine whether or not pausing bears any relation to the rate of production and to evaluate the accuracy of some methods for its calculation. Rate of production will hereafter be referred to as "rate" for the sake of simplicity.

Hays and Sanborn (*5*), in presenting different measurements of rate, state that, although the most intimate correlation of winter production with any of the four measures of intensity which they discuss is found to exist with the "net winter rate of laying", "mean size of winter clutch" is the measure to be preferred. In a later paper (*6*) the same authors claim that the average yearly clutch size is a more accurate criterion of intensity than any short-time measure. "The net winter rate of laying" is defined as the quotient of the total number of eggs from the first pullet egg to March 1 divided by the number of days in the same period less nonproductive periods of 4 or more consecutive days from November 1 to March 1.

Jull (*7*) proposes a different measure. For evaluation of rate, he uses the number of eggs in the same period studied by Hays and Sanborn expressed as percentage of the total number of days involved. It may be seen that the fundamental difference in the viewpoints of Jull and of Hays and Sanborn lies in the fact that the former evidently ascribes the occurrence of winter pauses to low rate, while the latter consider pause and rate to be separate factors. Knox, Jull, and Quinn (*8*) also state that the best measure of rate is the production to March 1 as percentage of total number of days involved.

A review of the available literature shows that no evidence as to the nature of a parallel condition of pause during the spring months has been presented. It is probably of some interest to determine whether or not spring pause is related to winter pause, as well as to investigate the relationship of these conditions to rate.

Such a study was undertaken with the foregoing points in view. Statistical constants and correlation of various factors in pausing and nonpausing groups of birds were calculated in the hope of throwing further light on the question.

[1] Received for publication June 13, 1935; issued February, 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 47.

METHODS

Two periods of production were studied, namely, the winter period, extending from November 1 to February 28, and the spring period, from March 1 to June 28, each exactly 120 days long.

In order to exclude the effect of pausing, broodiness, and late maturity, the rate of production was defined as the number of eggs laid during a period multiplied by 100 and divided by the number of days in the period (120), less the number of days paused and broody. The definition of pause was adopted arbitrarily as 7 or more consecutive nonproductive days. In the case of winter rate, the denominator was further reduced by subtraction of the number of days from November 1 to the date of the first egg, when the latter fell after November 1. This measurement is similar to the one called by Hays and Sanborn (*5*) net winter rate of laying. It may be seen that the conception of rate introduced here is more closely related to the measure of rate by the mean clutch size, as proposed by Hays (*2*), than to the method of Jull (*7*), which does not take nonproductive periods into consideration when calculating rate.

The birds included in this study were selected from the general trapnested Single Comb White Leghorn flock of the Poultry Division hatched in March and April, on the basis of the following considerations: (1) Only birds which were alive on October 1 of the year following the year of hatch were used; (2) birds which produced less than 30 eggs in either of the two periods studied were eliminated; (3) only birds which commenced laying on or before November 15 of the pullet year were included in the group studied.

Thus the population used was a strictly selected one, and it must be understood that any conclusions arrived at are applicable only to birds conforming to the standards set above. It was felt that a better understanding of the nature of pauses and their relation to the rate of production could be obtained if the study were restricted to birds of the foregoing type. The reasons for this are fairly obvious: Requirement 1 aims to eliminate from consideration the birds which, although apparently healthy, were under the influence of some pathological condition which led to their death before the expiration of the first laying year. Requirement 2 was supplementary to the first one, in the sense that it tended to eliminate birds with abnormal production records. Although in the third year of study birds of that type were killed and examined for evidence of pathological conditions, and were thus eliminated by requirement 1, in the first 2 years no such autopsies were made. The second requirement was therefore introduced to guard against inclusion of diseased birds. Finally, the third requirement served to eliminate part of the possible influence exercised by differential sexual maturity on the factors studied.

PRESENTATION OF DATA

The total number of birds answering the above requirements in the 3 years of study was 578. These were divided into 4 groups within each year on the basis of the pauses that they exhibited. Thus 12 groups were obtained altogether, 3 groups of each of the following: Birds which paused during the winter period only (WP group), birds which paused only in the spring (SP group), birds which paused both during the winter and spring (WSP group), and birds which did

not pause at all (NP group). The distribution of these 4 groups for each of the 3 years (F, G, and H series, respectively) is shown in table 1. A gradual change in the proportion of pausing and nonpausing birds can be seen from the figures presented.

TABLE 1.—*Birds used and percentage distribution on a pause basis*

Pause	F series, hatched in 1931		G series, hatched in 1932		H series, hatched in 1933	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Winter	116	58.0	104	46.4	54	35.1
Spring	11	5.5	20	8.9	13	8.4
Winter and spring	45	22.5	44	19.6	16	10.4
Nonpause	28	14.0	56	25.0	71	46.1
Total, 578 birds	200	100.0	224	99.9	154	100.0

The comparatively rapid drop in the percentage of winter-pausing birds, as contrasted with the slow decrease in proportion of the spring-pausing group affords the first indication that the two conditions are separate. A further indication of this was obtained by calculating the coefficients of association between winter and spring pause (*9, p. 38*). These coefficients for the 3 years were found to be −0.006, +0.085, and +0.236, respectively, indicating that any association present is due to mere fluctuations of sampling.

TABLE 2.—*Statistical constants for production and rate of the F, G, and H series of birds shown in table 1*

Production or rate	WP group [1]		SP group [1]	
	Mean	Standard deviation	Mean	Standard deviation
Series F:				
Winter production	49.930±0.751	11.99±0.53	72.680±1.903	9.36±1.35
Spring production	89.330± .645	10.30± .45	69.950±3.285	16.16±2.33
Winter rate	62.300± .533	8.52± .37	61.090± .951	4.68± .67
Spring rate	74.975± .536	8.56± .38	69.275±1.858	9.14±1.32
Series G:				
Winter production	55.260± .982	14.85± .70	76.000±1.671	11.08±1.19
Spring production	88.920± .726	10.99± .52	69.000±2.359	15.64±1.67
Winter rate	64.755± .583	8.82± .41	64.000±1.315	8.72± .93
Spring rate	76.615± .554	8.38± .39	70.250±1.927	12.78±1.37
Series H				
Winter production	60.430±1.339	14.59± .95	85.845±2.760	14.76±1.95
Spring production	89.310±1.169	12.73± .83	58.350±3.325	17.78±2.35
Winter rate	68.200± .378	4.12± .27	71.130±1.150	6.15± .81
Spring rate	76.170± .536	5.84± .38	72.770±1.122	6.00± .79

Production or rate	WSP group [1]		NP group [1]	
	Mean	Standard deviation	Mean	Standard deviation
Series F:				
Winter production	49.610±1.414	14.06±1.00	74.500±1.320	10.35±0.93
Spring production	69.610±1.400	13.92± .99	90.210±1.047	8.21± .74
Winter rate	61.780± .794	7.89± .56	63.070±1.000	7.84± .71
Spring rate	70.665± .890	8.85± .63	75.570± .678	5.32± .48
Series G:				
Winter production	56.090±1.548	15.22±1.10	78.070± .711	7.89± .50
Spring production	67.230±1.752	17.23±1.24	88.960± .992	11.01± .70
Winter rate	63.705± .860	8.46± .61	66.955± .567	6.29± .40
Spring rate	70.865±1.211	11.91± .86	74.770± .798	8.86± .57
Series H:				
Winter production	59.500±1.866	11.07±1.32	82.390± .722	9.02± .51
Spring production	72.000±2.632	15.61±1.86	9[illegible].240±1.316	16.45± .94
Winter rate	66.060±1.163	6.90± .82	69.745± .506	6.33± .36
Spring rate	72.000±1.635	9.70±1.15	75.240± .594	7.42± .42

[1] WP=winter pause; SP=spring pause; WSP=both winter and spring pauses; NP=no pause.

Table 2 presents the statistical constants for the 12 groups of birds for winter production, spring production, winter rate, and spring rate. This table gives the basic data from which differences between groups were calculated. Since there is a possible effect of environment and managemental practices, differences between means were determined only within each series; table 3 presents these. In all of the tables probable and not standard errors are used.

TABLE 3.—*Differences between means of winter and spring production and rates for birds as calculated from data in table 2*

WINTER PRODUCTION

[Significant differences are indicated in boldface and borderline differences in italic]

Groups[1]	F series	G series	H series
NP to SP	1.820±2.316	2.070±1.816	−3.455±2.853
NP to WP	**24.570±1.519**	**22.810±1.212**	**21.960±1.521**
NP to WSP	**24.890±1.934**	**21.980±1.703**	**22.890±2.001**
SP to WP	**22.750±2.046**	**20.740±1.938**	**25.415±3.068**
SP to WSP	**23.070±2.371**	**19.910±2.278**	**26.345±3.332**
WP to WSP	.320±1.601	−.830±1.833	.930±2.297
SPRING PRODUCTION			
NP to WP	0.880±1.230	0.040±1.229	0.930±1.760
NP to SP	**20.280±3.448**	**19.960±2.559**	**31.890±3.576**
NP to WSP	**20.600±1.748**	**21.730±2.013**	**18.240±2.943**
WP to SP	**19.380±3.349**	**19.920±2.468**	**30.960±3.525**
WP to WSP	**19.720±1.541**	**21.690±1.897**	**17.310±2.880**
SP to WSP	.340±3.571	1.770±2.939	*−13.650±4.241*
WINTER RATE			
NP to SP	1.980±1.380	2.955±1.432	−1.385±1.257
NP to WP	.770±1.133	2.200± .813	1.545± .632
NP to WSP	1.290±1.277	*3.250±1.030*	3.685±1.268
SP to WP	−1.210±1.000	−.755±1.438	2.930±1.211
SP to WSP	−.690±1.239	.295±1.571	*5.070±1.636*
WP to WSP	.520± .956	1.050±1.039	2.140±1.223
SPRING RATE			
NP to WP	0.595± .864	−1.845± .969	−0.930± .800
NP to SP	*6.295±1.978*	4.520±2.086	2.470±1.270
NP to WSP	**4.905±1.119**	3.905±1.450	3.240±1.740
WP to SP	5.700±1.934	*6.365±2.005*	3.400±1.243
WP to WSP	**4.310±1.039**	**5.750±1.332**	4.170±1.720
SP to WSP	−1.390±2.060	−.615±2.276	.770±1.983

[1] For explanation of symbols see footnote to table 2.

Table 3 shows that, as would be expected, occurrence of a pause or pauses in the winter period reduced the winter egg production by 19.9 to 26.3 eggs in the various groups. The groups which showed spring pause did not differ significantly in winter production from the non-spring-pause groups. Likewise there was no significant difference in winter production between the winter-pause birds and those birds which besides the winter pause also exhibited a spring pause.

Similarly, table 3 shows that spring production is reduced by the occurrence of spring pause by 17.3 to 31.9 eggs, while winter pause does not seem to have any effect on the spring production. The only exception to this is the case of the winter- and spring-pause group of

the H series. While still significantly below the nonpause and winter-pause groups in spring production, this group shows a mean spring production higher by 13.65 eggs than the group which paused during the spring only. This figure is slightly more than three times its probable error and as such approaches the borderline limit of statistical significance.

Table 3, which also shows differences in winter rate between the various groups, is of interest since it discloses that winter rate as here calculated is not affected by the occurrence of winter pause. Only two of the differences at all approach statistical significance and even these are less than 3.5 times the probable error. In 3 of the 12 cases, the group showing winter pause has a higher winter rate than the corresponding nonpause groups. This seems to be an indication that winter pause should not be considered merely as a manifestation of low rate.

So far as spring pause is concerned, it may be seen from table 3 that in some cases this condition appears to affect the rate of spring production. Of the 12 differences in spring rate between the groups not pausing in the spring and the groups exhibiting a spring pause, 3 are statistically significant while 2 more are below 3.5 but above 3.0 times their respective probable errors. There is, therefore, a possibility of an association between spring rate and spring pause. The coefficients of association between low spring rate and spring pause were found to be +0.492, +0.344, and +0.286 for the F, G, and H series, respectively. Thus it seems that there is a difference in the nature of the winter and spring pauses. Should any genetic interpretation of winter pause, such as that of Hays (*1*), be accepted, one must perforce conclude that the spring-pause condition, if genetic in nature, is governed by factors other than those controlling winter pause.

In order to determine whether or not pauses have an effect on the production in periods immediately preceding and immediately following them, the rates of production for the 15 days before and for the 15 days after a pause were calculated. These values are presented in table 4 together with the rate for the total winter or spring

TABLE 4.—*Rate of production for 15 days preceding and for 15 days following a pause*

Group [1]	Winter rate			Spring rate		
	Pre-pause	Total period	Post-pause	Pre-pause	Total period	Post-pause
Series F:						
WP	58.96	62.30	58.83			
SP				61.23	69.28	77.81
WSP	57.23	61.78	58.96	68.47	70.67	67.40
Series G:						
WP	60.70	64.76	54.83			
SP				69.33	70.25	66.67
WSP	58.81	63.71	50.36	68.23	70.87	65.17
Series H:						
WP	64.70	68.20	67.27			
SP				73.34	72.27	62.23
WSP	62.65	66.06	66.23	69.60	72.00	67.54

[1] For explanation of symbols see footnote to table 2.

period, as the case may be. The rate both before and after a pause seems to be somewhat depressed except in a few cases. This reduction in rate preceding and following the pause may be an expression of the factors producing the pause and not a true manifestation of factors for rate, at least in the case of winter rate, since, as shown in table 3, the winter rate of pausers for the whole period does not differ significantly from the winter rate of nonpausers.

COEFFICIENTS OF CORRELATION

Coefficients of correlation between the four factors studied were computed and are presented in table 5. The most significant points to be noted from these correlations are:

(1) The coefficients of correlation between winter rate and winter production are, as expected, somewhat higher in groups which did not pause in the winter than in groups which did.

TABLE 5.—*Coefficients of correlation in the different series as calculated from the data in table 3*

Factors correlated	F series	G series	H series
Group WP:[1]			
Winter production and winter rate	0.288±0.058	0.460±0.052	0.634±0.055
Winter production and spring production	.101± .063	.227± .063	.445± .074
Winter production and spring rate	.179± .061	.327± .059	.388± .078
Winter rate and spring production	.327± .056	.478± .051	.431± .075
Winter rate and spring rate	.422± .052	.572± .044	.592± .060
Spring production and spring rate	.839± .019	.812± .021	.818± .030
Group SP:[1]			
Winter production and winter rate	.897± .040	.927± .021	.955± .016
Winter production and spring production	.726± .096	.375± .130	.122± .184
Winter production and spring rate	.367± .176	.548± .106	.411± .155
Winter rate and spring production	.660± .114	.411± .126	.149± .183
Winter rate and spring rate	.261± .189	.626± .086	.381± .160
Spring production and spring rate	.024± .124	.828± .047	.477± .144
Group WSP:[1]			
Winter production and winter rate	.411± .084	.614± .064	.758± .063
Winter production and spring production	.241± .095	.112± .101	.253± .139
Winter production and spring rate	.026± .101	.392± .086	.058± .149
Winter rate and spring production	.354± .088	.059± .102	.225± .141
Winter rate and spring rate	.354± .088	.363± .089	.211± .142
Spring production and spring rate	.604± .064	.746± .045	.888± .031
Group NP:[1]			
Winter production and winter rate	.881± .028	.896± .018	.720± .039
Winter production and spring production	.294± .116	.434± .073	.367± .069
Winter production and spring rate	.389± .107	.472± .070	.376± .069
Winter rate and spring production	.322± .114	.443± .072	.573± .054
Winter rate and spring rate	.401± .107	.455± .071	.531± .057
Spring production and spring rate	.882± .028	.927± .013	.965± .006

[1] For explanation of symbols see footnote to table 2.

(2) The correlation coefficients between spring rate and spring production are higher in the nonspring-pause groups than in the spring-pause groups, although the differences here are not so marked as in the case of the winter period. The occurrence of broody periods in the summer months in groups which did not show any pause is probably responsible for this.

(3) The magnitude of the correlation coefficients between winter and spring production varies from $+0.101 \pm 0.063$ for the WP group of the F series to $+0.726 \pm 0.096$ for the SP group of the same series. No particular regularity can be observed in the variation of this coefficient.

(4) The correlation between winter and spring rate also shows some variation, the lowest coefficient being $+0.211 \pm 0.142$ for the WSP

group of the H series, and the highest $+0.626 \pm 0.086$ for the SP group of the G series. It is a fact of great importance that, in all cases except two, winter rate is more closely correlated with spring rate than is winter production with spring production. The two exceptions represent populations of 11 (SP group, F series) and of 16 (WSP group, H series) respectively. It should also be noted that the correlation between spring rate and winter rate may be somewhat reduced in the total population as a result of spring pause.

Figure 1, based on the total population of 578 birds, illustrates the regression of spring rate on winter rate. It is definitely linear, the difference between the correlation ratio and correlation coefficient being, according to Blakeman's test, 0.017 ± 0.007. Hays and Sanborn (*6*), however, found that the correlation between winter- and spring-clutch size was curvilinear to a small degree.

It may be seen from the slope of the line in figure 1 that the percentage of improvement in spring rate over winter rate is greater in the birds with the lower winter rate. Birds exhibiting a winter rate of about 80 percent and over do not show any increase of rate in the spring, and may even exhibit a decline. On the whole, it may

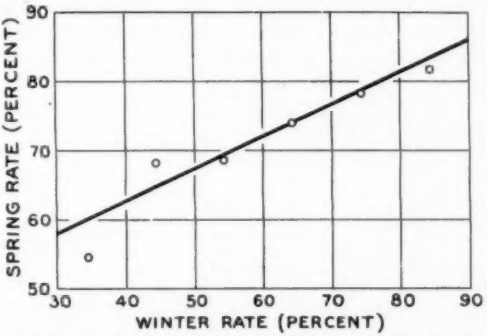

FIGURE 1.—Regression of spring rate on winter rate; $SR = 44.256 + 0.463\ WR$.

be seen that there is a possibility that the winter and the spring rates are governed by the same genetic factors, their expression depending on environmental conditions.

It may be reiterated here that two views on the matter are possible. One view, that of the Beltsville workers (*7*, *8*), is that pauses are a reflection of a low rate, the other, held by Hays and Sanborn (*5*, *6*), is that winter pause is a separate entity. The evidence presented here may be interpreted to mean that pauses, whether genetically or environmentally determined, should be excluded in any method of measuring rate.

The effect of inclusion of pause periods in the calculation of rate may be observed in table 6. Series G is used as the one with the largest number of individuals. The winter and spring rates are shown for the various groups as calculated by two methods. The Beltsville method consists in dividing the number of eggs in a period $\times 100$ by the total number of days, while in the California modification of the Massachusetts method the days of pause and of broodiness are first subtracted from the denominator.

TABLE 6.—*Comparison of methods of measuring rate of production in various groups [1] of the G series by Beltsville and California methods*

Rate and method	WP group	SP group	WSP group	NP group
Winter:				
Beltsville	46.05	63.33	46.74	65.06
California	64.76	64.00	63.71	66.96
Spring:				
Beltsville	74.10	57.50	56.03	74.13
California	76.62	70.25	70.87	74.77

[1] For explanation of symbols see footnote to table 2.

According to the Beltsville method, the birds in the WSP and the NP groups are, respectively, low and high for both periods. In the WP group the birds are low for winter rate but high for spring rate, while in the SP group the reverse is true. On the other hand, according to the California method, the birds in each of the groups show the same type of rate irrespective of the season for which rate is calculated. By using the regression line in figure 1 it is then possible to approximate the rate of one period from information on the rate of the other. If rate is a character controlled by the same factors throughout the year, this evidence indicates that there is no justification for any method of measuring rate which does not exclude the pausing periods from consideration.

The absence of any relationship between winter rate as here calculated and some of the other factors which may affect production is shown by the following coefficients of correlation (WP group, G series) between—

Winter rate and—	
Date of hatch	-0.011 ± 0.066
Date of first egg	$-.005 \pm .066$
Age at first egg	$-.046 \pm .066$
Winter pause length	$-.078 \pm .066$

While Hays (*3*) has found that his nonbroody line of Rhode Island Reds had a mean winter production higher by about 12 eggs than his broody line, in the case of the Single Comb White Leghorns here studied, there was no difference between broody and nonbroody birds in rate of winter production, but there was some difference in spring rate. Table 7, illustrating this point, shows that the broody birds had a higher rate of spring production than the nonbroody birds. Because of the small number of broody birds, it is not possible to check the significance of this observation by the usual methods.

TABLE 7.—*Rate of production of broody and of nonbroody birds*

Series or group [1]	Total population	Broody	Winter rate		Spring rate	
			All birds	Broody birds	All birds	Broody birds
		Number				
F	200	11	62.225	62.400	73.775	75.091
WP in G	104	10	64.755	64.400	76.615	80.500
G	224	25	64.745	66.640	74.480	80.320
H	154	8	68.980	69.500	75.280	80.500

[1] For explanation of symbols see footnote table 2.

CONCLUSIONS AND SUMMARY

A study of the relation of pauses to the rate of egg production was made on a selected population of 578 Single Comb White Leghorns in their pullet year. The birds were selected for absence of pathological conditions and for fairly uniform early sexual maturity. Three series of birds, hatched in 3 consecutive years, were represented in the total population and each series was treated separately. Analyses of differences in the rate of production between pausing and nonpausing birds, as well as a study of correlations between rate and number of eggs laid during different periods led to the following major conclusions:

(1) The condition of winter pause is causally distinct from that of spring pause and is entirely separate from it.

(2) The view that winter pause is merely a manifestation of low winter rate is untenable.

(3) Spring pause may be associated with low spring rate, but no conclusive evidence on this point is as yet available.

(4) Spring rate shows a linear regression on winter rate and an increase over it when the latter is below 80 percent. Evidence on hand points to the possibility that the same genetic factors control both winter and spring rate of production.

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THE EFFECT OF HOMOGENIZATION ON SOME OF THE CHARACTERISTICS OF MILK FAT [1]

By I. A. GOULD and G. M. TROUT, *research assistants in dairy husbandry, Michigan Agricultural Experiment Station* [2]

INTRODUCTION AND REVIEW OF LITERATURE

The adaptation of the homogenization process to the market-milk industry has been confined largely to pasteurized milk, since raw milk was found to develop a rancid flavor within a few hours after being homogenized. The rapid development of rancidity in homogenized raw milk and the absence of a similar development in milk pasteurized prior to homogenization has been noted by several investigators, including Dorner and Widmer (*5*),[3] Halloran and Trout (*6*), Doan and Minster (*4*), and Babcock (*2*). Dorner and Widmer (*5*) attributed the development of the rancid flavor to lipase action resulting in the splitting of the fat to give free fatty acids. This view has been generally accepted by other investigators. The associates of Rogers (*8*) had earlier pointed out that the production of free fatty acids in some dairy products is due primarily to hydrolytic action of enzymes or the action of free acids in the product in the presence of water. These authors state further that the production by hydrolysis of some of the lower fatty acids, such as butyric, caproic, and others, are responsible for the rancid flavor and odors.

Dorner and Widmer (*5*), and Halloran and Trout (*6*), working independently, observed an increase in titratable acidity of homogenized raw milk, the increase appearing immediately after homogenization and varying directly with the pressure used. Pasteurization before homogenization prevented the occurrence of any increase in acidity. Doan and Minster (*4*) secured similar results by pH measurements.

The decrease in the surface tension of homogenized raw milk, as noted by Halloran and Trout (*6*), was ascribed by Doan (*3*) to be the result of the liberation of some of the lower fatty acids from the glyceride, and an increase in their concentration at the surface of of the milk.

The associates of Rogers (*8*), Lewkowitsch (*7*), and others give the normal ranges of characteristic milk-fat constants, such as refractive index, Reichert-Meissl number, Polenske number, and acid value. However, no information is available regarding the effect of homogenizing either raw or pasteurized milk on these values. According to the former authors, the normal range of the refractive index of milk fat at 40° C. is from 1.4527 to 1.4566. Lewkowitsch (*7, v. 1, p. 124*) studied pure fatty acids and found the indexes of butyric and caproic acids at 20° C. to be 1.39906 and 1.41635, respectively.

[1] Received for publication July 22, 1935; issued February 1936. Journal article no. 223 (n. s.) from the Michigan Agricultural Experiment Station.

[2] The authors wish to express their appreciation to Prof. C. D. Ball, Chemistry Department, Michigan State College, for valuable suggestions and criticisms relative to this study.

[3] Reference is made by number (italic) to Literature Cited, p. 56.

SCOPE OF INVESTIGATION

Although suggestions have been made that increases in titratable acidity, decreases in the hydrogen-ion concentration, and development of rancidity in homogenized raw milk were due to changes in the fat, all evidence for these assumptions has been more or less indirect in that the measurements have been made upon the milk rather than upon the fat itself. In this connection it appeared of interest to determine the actual chemical changes in the fat brought about by homogenization both of raw and of pasteurized milk, and to determine the extent to which the fat is changed in the development of rancidity. To secure such information, a study of changes in some of the fat constants appeared to offer the best possibilities. The fat constants chosen for examination were as follows: The Reichert-Meissl number, the Polenske number, the acid degree, and the refractive index.

PROCEDURE

Raw milk having a fat test of approximately 3.5 percent was used for the experiments. During each of the five trials run, the milk was divided into four lots and processed with commercial equipment, as follows:

Lot 1. Pasteurized at 145° F. for 30 minutes.

Lot 2. Pasteurized at 145° F. for 30 minutes, and then homogenized at 1,500 pounds pressure per square inch.

Lot 3. Warmed to 100° F., homogenized at 1,500 pounds pressure, and immediately thereafter pasteurized at 145° for 30 minutes. About 10 to 15 minutes was usually required to reach the pasteurizing temperature after homogenization.

Lot 4. Homogenized at 100° F., cooled to about 55° over a surface cooler, and then stored at 35° to 40° for 24 hours. After storage, the milk was pasteurized, as in the previous lots.

Following the processing of the lots, samples were taken, cooled, and the pH value and titratable acidity of the milk determined. The pH value was obtained electrometrically with a Leeds-Northrup portable acidity meter using a bright platinum foil electrode and quinhydrone for the determinations. The milk was then separated, and the cream churned. The fat obtained was washed several times with water and melted in a hot-water bath. It was further purified by filtering through a paper filter to eliminate curd and water. The purified fat obtained was used for the determination of the Reichert-Meissl number, the Polenske number, the acid degree, and the refractive index. An Abbé refractometer was used, according to the directions of the Association of Official Agricultural Chemists (*1*).

RESULTS

THE EFFECT OF HOMOGENIZATION ON THE TITRATABLE ACIDITY AND HYDROGEN-ION CONCENTRATION OF THE MILK

As mentioned previously, several investigators observed the effect on the titratable acidity and the hydrogen-ion concentration when raw and pasteurized milk were homogenized. These published results showed that the homogenization of pasteurized milk had no effect on the hydrogen-ion concentration or acidity, whereas the homogenization of raw milk increased the acidity and decreased the hydrogen-ion concentration. A verification of these results was

secured in this study, but the data are not included inasmuch as they are a repetition of what has formerly been thoroughly studied. It suffices to say that in the five trials made, no appreciable differences occurred either in the titratable acidity or in the hydrogen-ion concentration when the milk was homogenized following pasteurization, but a noticeable change in both of these values was observed when the milk was homogenized raw and then pasteurized. These changes were greater when 24 hours had elapsed between homogenization and pasteurization. During this period, the titratable acidity increased from an average of 0.17 to an average of 0.20 percent, calculated as lactic acid, while the hydrogen-ion concentration value decreased from 6.58 to 6.44. The determination of titratable acidity, calculated as lactic acid, appeared to be as accurate as hydrogen-ion concentration measurements in detecting acidity changes in the milk due to homogenization.

THE EFFECT OF HOMOGENIZATION OF MILK ON THE REICHERT-MEISSL AND POLENSKE NUMBERS OF THE FAT

The data secured in the study of the influence of homogenization of raw and pasteurized milk on the Reichert-Meissl and Polenske values of the butterfat are presented in table 1.

TABLE 1.—*The effect of homogenizing raw and pasteurized milk upon the Reichert-Meissl and Polenske numbers, the refractive index, and the acid degree of the butterfat*

REICHERT-MEISSL NUMBER

Trial no.	Pasteurized, unhomogenized	Pasteurized, homogenized	Raw, homogenized	Raw, homogenized, stored 24 hours
1	29.68	28.90	30.21	29.12
2	31.25	30.94	31.44	31.00
3	30.14	30.00	29.81	29.24
4	30.97	30.66	30.80	30.65
5	28.18	28.11	27.93	27.79
Average	30.04	29.72	30.04	29.56

POLENSKE NUMBER

1	3.27	3.27	3.25	3.18
2	2.84	2.83	2.93	2.85
3	2.07	2.07	1.89	1.82
4	2.88	2.90	2.80	2.76
5	1.89	2.00	1.92	1.93
Average	2.59	2.63	2.56	2.51

REFRACTIVE INDEX

1	1.4542	1.4541	1.4541	1.4540
2	1.4552	1.4552	1.4551	----------
3	1.4552	1.4551	1.4550	1.4551
4	1.4552	1.4552	1.4551	1.4548
5	1.4551	1.4550	1.4550	1.4552
Average	1.4550	1.4549	1.4545	1.4548

TABLE 1.—*The effect of homogenizing raw and pasteurized milk upon the Reichert-Meissl and Polenske numbers, the refractive index, and the acid degree of the butterfat*—Continued

ACID DEGREE [1]

Trial no.	Pasteurized, unhomogenized	Pasteurized, homogenized	Raw, homogenized	Raw, homogenized, stored 24 hours
1	0.72	0.74	2.65	9.86
2	.32	.33	2.55	
3	.67	.60	[2] 3.73	11.20
4	.50	.50	2.53	14.40
5	.65	.66	2.70	6.12
Average	.572	.566	2.608	10.395

[1] Acid degree is expressed as the cubic centimeters of N/1 NaOH required to titrate the free fatty acids in 100 g of fat.
[2] Not included in averages.

The averages of these trials vary but do not show any general trend in changes of these constants. The data show no great differences between the fat from the homogenized raw samples and that from the original unhomogenized pasteurized milk. The Reichert-Meissl and Polenske titrations decreased only 0.48 and 0.08 cc, respectively, in the 24-hour raw sample. Such changes appear negligible.

THE EFFECT OF HOMOGENIZATION OF MILK ON THE REFRACTIVE INDEX OF THE FAT

The results of the study of homogenization as it affects the refractive index of the milk fat are also presented in table 1. As shown in this table, homogenization of the raw or of the pasteurized milk had no marked influence on the refractive index. A slight decrease in the indexes of the fat from the raw samples is shown by the average of the trials, but the change is so slight as to appear of no value.

THE EFFECT OF HOMOGENIZATION OF MILK ON THE ACID DEGREE OF THE FAT

Although none of the fat constants reported in table 1 appeared to be affected by homogenization, the acid degrees of the fat were materially changed. Table 1 gives the values secured. The data show that the average acid degree of the fat of the raw homogenized milk immediately after processing was approximately 4 times that of the pasteurized milk, whereas after the raw homogenized milk had been stored 24 hours the average acid degree of the fat was 18 times that of the pasteurized milk.

The acid values secured were practically constant between the two pasteurized samples, indicating that there was no liberation of fatty acids when the milk was pasteurized prior to homogenization.

The acid degree of the fat in the raw homogenized sample in trial no. 3 showed scarcely any increase as it did in the other four trials, and, because of this deviation, the value was not included in the averages.

The values secured for the raw homogenized samples stored 24 hours varied greatly. The exact cause for these variations is not known.

THE EFFECT OF LONG STORAGE PERIODS ON THE TITRATABLE ACIDITY AND HYDROGEN-ION CONCENTRATION VALUES OF THE RAW HOMOGENIZED MILK AND ON THE ACID DEGREE OF THE FAT

Since the acid value was found to be so greatly affected when raw milk was homogenized, it was thought desirable to make a study of the changes in the free acids of the fat when the milk was stored for several days following homogenization. The raw milk was homogenized at 1,500 pounds pressure, cooled immediately over a surface cooler to 55° F., and stored at 35° to 40°. Portions of this milk were taken at daily intervals, pasteurized, and the fat studied. The control sample was pasteurized but not homogenized.

Titratable acidities and hydrogen-ion concentration measurements were also made on the milk after varying periods of storage. The values of these latter determinations are given in table 2 and show a marked increase in titratable acidity and increase in hydrogen-ion concentration in the milk during the 5-day storage period.

TABLE 2.—*The effect of storing for varying periods of time, the titratable acidity, and hydrogen-ion concentration of raw homogenized milk*

Period after homogenization (hours)	Trial no. 1		Trial no. 2		Trial no. 3		Average	
	Acidity	pH	Acidity	pH	Acidity	pH	Acidity	pH
	Percent		*Percent*		*Percent*		*Percent*	
Control	0.182	6.42	0.155	6.50	0.145	6.38	0.161	6.43
¼–½	.190	6.38	.170	6.48	.155	6.35	.172	6.40
24	.240	6.24	.200	6.28	.185	6.32	.208	6.28
48	.245	6.25	.240	6.28	.185	6.32	.223	6.28
72	.255	6.19	.235	6.28	.190	6.32	.227	6.26
96	.255	6.14	.238	6.20	.195	6.30	.229	6.21
120	.265	6.17	.245	6.15	.200	6.26	.237	6.19

The data showing the effect of the varying periods of storage on the acid degree of the fat are presented in table 3. These figures show the tremendous increase of free acids in the homogenized raw milk during each of the storage periods. Even at the end of the fifth day the liberation of the acids continued rapidly.

TABLE 3.—*The effect of storing for varying periods of time on the acid degree of the fat of raw homogenized milk*

Period after homogenization (hours)	Acid degree of the fat				Average increase
	Trial no. 1	Trial no. 2	Trial no. 3	Average	
					Percent
Control	0.595	0.645	0.460	0.566	0
¼–½	4.390	3.850	2.515	3.585	533
24	11.200	9.960	8.600	9.920	1,652
48	14.690	13.370	8.560	12.207	2,056
72	16.000	14.980	10.300	13.760	2,331
96	18.500	15.200	10.870	14.856	2,524
120	18.400	18.010	12.640	16.350	2,788

The greatest daily change in the fatty-acid values occurred during the first 24 hours of storage, the increase amounting to an average of 1,652 percent during this time. Furthermore, the acid value of the

fat increased on an average of 533 percent within a few minutes after homogenization.

The increase in the degree of free acids in the fat, and in titratable acidity, and the decrease of the pH value are shown in figure 1. In this figure the pH values were plotted as hydrogen-ion concentration [H+] so as to form a curve which may be compared as to slope with the curve of the acid value.

Figure 1 shows the rapid increase in the free fatty acids during the first 24 hours, or even during the first few minutes, after homogenization. The speed of the splitting of the fatty acids from the glycerol

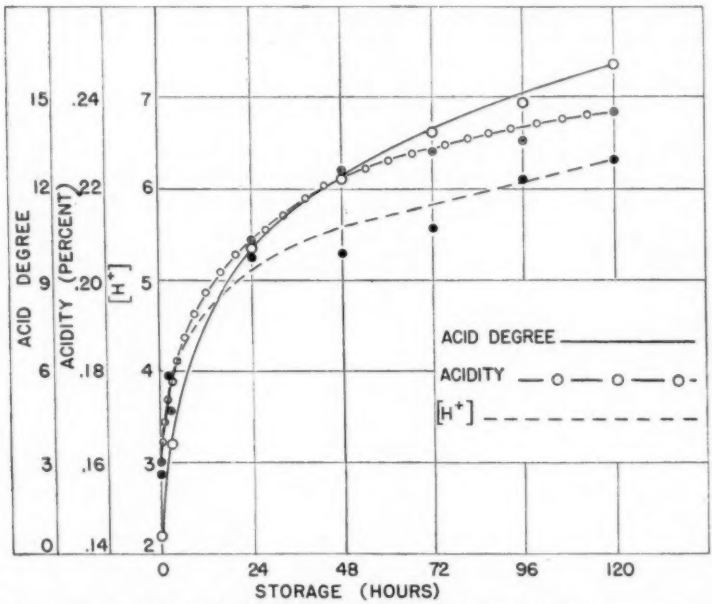

FIGURE 1.—Changes in the acid degree of the fat, the titratable acidity, and the hydrogen-ion concentration of raw homogenized milk. The units indicating [H+] should be multiplied by 10^{-7} to express accurate concentration. The acidity is expressed as the percentage of lactic acid, and the acid degree as the number of cubic centimeters of N/1 NaOH required to titrate the free fatty acids in 100 g of fat.

by enzymic action apparently proceeds at a high velocity when the fat globules are first broken by the homogenizing process into smaller globules, thus exposing more surface to the splitting action.

Figure 1 also shows that the titratable-acidity measurements of the milk followed increases in free acids of the fat somewhat more closely than the pH values. Since only three trials supplied data for this graph it may be presumptous to assume that this difference will always occur. It appears safe to say, however, that determinations of rancidity development in milk may be made as satisfactorily with titration means as with potentiometric methods.

DISCUSSION

Of the four fat constants investigated in the present study only the acid value showed distinct changes as a result of homogenization of raw milk. Homogenization of pasteurized milk did not cause any appreciable changes in the Reichert-Meissl number, the Polenske number, the refractive index, or the acid degree.

If only the liberation of the lower fatty acids occur in the fat of homogenized raw milk, no changes would be expected in the Reichert-Meissl or Polenske numbers since the amount of soluble volatile and insoluble volatile acids would naturally remain constant regardless of whether the acid was in the free or combined state. It is likewise obvious that during the determination of these two constants, the lower fatty acids must necessarily be freed.

A decrease in the refractive index of milk fat might be expected to occur with high concentrations of free acids, when consideration is given to the refractive indices of pure fatty acids. The average refractive index of the fat in this study was 1.4548 at 40° C., whereas Lewkowitsch (*7, v. 1, p. 124*) gave a value of 1.39906 at 20° for pure butyric acid. Correcting the milk-fat index of 1.4549 at 40° to 20° by using the temperature correction formula $R=R'+0.00038\ (T'-T)$ as given by the Association of Official Agricultural Chemists (*1*), the index of refraction of the fat is increased to 1.4625. This correction makes even larger the difference between the indexes of the butyric acid and the fat, indicating that the presence of any appreciable amount of butyric acid would have a marked tendency to lower the index of refraction of milk fat. In the trials herein reported, however, no great differences were detected even though the acid degree increased on an average from 0.572 to 10.40 during a 24-hour holding period. The averages of the five trials show a slight decrease in the refractive index of the fat in the raw samples, but the decrease is so small as to appear negligible. A greater decrease than this would be expected with an acid degree of 10.37, or the presence of 0.912 percent n-butyric acid.

In this connection, however, the refractive index of the glycerol must not be overlooked. Lewkowitsch (*7, v. 3, p. 402*) gives values for pure glycerol secured both by Strohmer and Lenz which are higher than the index values of butterfat. The refractive index of glycerin at 20° C. is approximately 1.4727. Therefore, when butterfat is hydrolyzed, the slightly higher index of refraction of glycerin might tend to balance the lower values of the free fatty acids.

The interesting point in regard to the acid degrees of the fat taken from raw homogenized milk after varying periods of storage is the large increase in this value. The increase on an average of 2,788 percent over the control pasteurized sample after 5 days' storage illustrates the rate at which fat-splitting action proceeds even at low-storage temperatures. Calculations show that the 16.35 cc of N/1 NaOH required to titrate the free acids in 100 g of fat are equivalent to 1.4388 g of butyric acid. Assuming the butyric acid content of butterfat to be 3 percent, these values indicate that about 48 percent of the butyric acid is liberated at the end of the 5-day storage period. This value may be somewhat low if consideration is given to the possibility that some of the glycerol and butyric acid are lost

during the washing of the fat. Likewise, it is likely that other lower fatty acids in addition to butyric are affected by the fat-splitting process.

It is known that the homogenization process increases the surface of the fat globules about 4 to 6 times. The data obtained in this study show that the acid degree of the fat in the raw homogenized samples increased fourfold to sixfold within a few minutes after homogenization of raw milk. Perhaps the entire immediate increase in the acid degree following homogenization may be explained by the increase in the surface of the fat, thus permitting greater fat-splitting activity by the causative agent. Further increases may be due not only to enzymic action but to the presence of the free acids which hasten further hydrolysis of the fat.

From time to time rather distinct differences were observed between the speed with which the acid value increased in samples of milk with the same fat content. These differences may have been partly due to changes in storage temperatures but more likely were principally caused by variations inherent in the milk itself.

SUMMARY AND CONCLUSIONS

No appreciable differences in the Reichert-Meissl number, the Polenske number, the refractive index, or in the acid degree occurred in the fat when pasteurized milk was homogenized at 1,500 pounds pressure per square inch.

When raw milk was homogenized, the acid degree of the fat increased fourfold to sixfold within a few minutes.

The normal average acid degree of the fat (expressed in cubic centimeter N/1 NaOH per 100 g of fat) was 0.572. This value increased in raw milk to 2.608 immediately after homogenizing.

The greatest daily change in the acid degree of the fat of stored homogenized raw milk occurred during the first 24 hours when the titration of free acids with N/1 alkali increased on an average from 0.566 to 9.92 cc, a 1,652-percent increase.

Homogenized raw milk, stored for 5 days, required an average of 16.35 cc N/1 NaOH to titrate the free acids in 100 g of fat, or an equivalent of 1.4388 g of butyric acid.

The measurement of free fatty acids by titration of the fat appears to be a more accurate and more sensitive means of determining the rate of fat-splitting action than those determinations which may be made upon the milk.

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PERIDERM AND CORTEX COLOR INHERITANCE IN THE POTATO [1]

By F. A. KRANTZ, *associate professor of horticulture, in charge of vegetable gardening, Minnesota Agricultural Experiment Station, and collaborator, Division of Fruit and Vegetable Crops and Diseases, United States Department of Agriculture;* and HAROLD MATTSON, *assistant in horticulture, Minnesota Agricultural Experiment Station, and agent, Division of Fruit and Vegetable Crops and Diseases, United States Department of Agriculture*

INTRODUCTION

Red and blue tuber color in varieties of white-and yellow-fleshed potatoes (*Solanum tuberosum* L.) is due to pigment located in the periderm, in the peripheral cortex, or in both periderm and cortex. Krantz (*5*) [2] found the location of pigment to be specific for certain progenies. Location of pigment is not always indicated, and in general receives little attention in the contributions of Salaman (*10, 11*), Asseyeva (*1*), Sirks (*12*), Lunden (*7*), Black (*2*), and Rathlef (*9*). McIntosh (*8*) and Klapp (*3*) found, in systematic studies of potato varieties, that color of flowers was associated with color of periderm, but not with color of cortex. This paper presents data on the inheritance of periderm color and cortex color and also considers interaction between periderm and cortex color factors.

INHERITANCE OF RED COLOR IN THE PERIDERM

Color in the periderm (the cork or skin) may usually be recognized in portions of the periderm separated by light scraping from the underlying tissue of immature tubers.

Previous observations (*4, 5, 6*) have aided the formulation of a working hypothesis which assumes that red periderm color is due to the complementary action of three factors *E*, *S*, and *F*.

The frequencies of red-periderm and white-periderm individuals in progenies of 11 various selfed parents are recorded in table 1. The distribution suggests a relation of 3:1 in family no. 85, the progeny of a plant of unknown tuber color, and 9:7 in 3 families and 27:37 in 3 families, progenies of selfed seedlings which had red tuber color only in periderm of the eye region. These numerical relationships would be expected if three interacting factors were operating to produce red color in the periderm and if the respective parents were segregating for 1, 2, and 3 factor pairs.

The relation of 45:19 suggested by the frequencies in progenies of 3 red-periderm parents, the Triumph variety and seedlings nos. 5–10–1 and 5–14–8–1, would be expected if these parents were heterozygous for 3 pairs of interacting factors, 2 of which were duplicates.

[1] Received for publication July 16, 1935; issued February, 1936. Journal Series Paper No. 1370 of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 63.

TABLE 1.—*Periderm color in progenies of certain selfed potato plants*

Seedling or variety no.	Periderm color of parent	How derived	Progeny having indicated periderm color			Suggested factorial formula	Theoretical ratio of red to white
			Red	White	Total		
			Number	*Number*	*Number*		
85	Unknown	Observed	153	47	200	*EE SS Ff*	
		Calculated	150	50	200		3:1
41-2-2-3	Red eye [1]	Observed	125	95	220	*Ee Ss FF*	
		Calculated	123.75	96.25	220		9:7
8-4	do.[1]	Observed	94	89	183	*Ee Ss FF*	
		Calculated	103	80	183		9:7
12-2-22	do.[1]	Observed	75	43	118	*Ee Ss FF*	
		Calculated	66	52	118		9:7
5-3	do.[1]	Observed	213	252	465	*Ee Ss Ff*	
		Calculated	196	269	465		27:37
5-6	do.[1]	Observed	37	40	77	*Ee Ss Ff*	
		Calculated	32.5	44.5	77		27:37
41-2-7	do.[1]	Observed	30	56	86	*Ee Ss Ff*	
		Calculated	36	50	86		27:37
5-10-1	Red	Observed	223	89	312	*Ee Ss S's' FF*	
		Calculated	219.4	92.6	312		45:19
5-14-8-1	do	Observed	67	34	101	*Ee E'e' Ss FF*	
		Calculated	71	30	101		45:19
Triumph	do	Observed	252	102	354	*Ee E'e' Ss FF*	
		Calculated	249	105	354		45:19
5-14	Light red	Observed	35	26	61	*Ee E'e' Ss Ff*	
		Calculated	32	29	61		135:121

[1] Red confined to periderm of eye region.

The proportion of red to red-eye periderm color was 71 to 152 in family no. 5–10–1, in contrast to the proportions of 198 to 54 and 49 to 18 in progenies of the Triumph and no. 5–14–8–1. This difference suggests that the pair of duplicate factors segregating in seedling 5–10–1 is not the same as the pair of duplicate factors heterozygous in the Triumph and in no. 5–14–8–1.

Its light red-periderm color, the distribution of color in its progeny, and in the progeny of its second-generation seedling no. 5–14–8–1 are considerations which suggest that seedling no. 5–14 was heterozygous for 4 pairs of interacting factors, 2 of which were duplicates.

INHERITANCE OF CORTEX COLOR

Cortex color is assumed to be governed by four pairs of interacting genes. According to this working hypothesis, red-cortex color is considered to be due to the complementary action of factors *R*, *D*, and *C*, and blue cortex to result from the addition of a factor *P*.

INHERITANCE OF RED COLOR IN THE CORTEX

As recorded in table 2, the proportions of red-cortex individuals in families resulting from selfing the Flourball and Early Ohio varieties and in the progeny, no. 10.22, of a cross between the Early Ohio and a white-cortex seedling suggest that these varieties were heterozygous for two interacting factors for cortex color. A similar condition is indicated for Early Ohio seedling no. 41–17 by its color and by the proportion of red-cortex individuals in family no. 11.

When five cortex-color seedlings of family 11 were selfed, the behavior of faint-red cortex seedlings nos. 11–4 and 11–8 indicated that they may have been heterozygous for three pairs of interacting

cortex color factors. The behavior of seedling no. 11–4 in crosses recorded in tables 2 and 3 supports this conclusion, especially when considered in comparison with the behavior of light-red cortex seedling no. 11–3 in similar crosses.

TABLE 2.—*Cortex color in progenies of selfed red-cortex individuals and in progenies of certain red-cortex × white-cortex crosses*

Family no.	Parents and color	Progeny having indicated cortex color				Suggested factorial formula	Theoretical ratio of red to white
		How derived	Red	White	Total		
			Number	*Number*	*Number*		
7	Flourball (light red)	Observed	84	66	150	*CC Rr Dd*	
		Calculated	84	66	150		9:7
41	Early Ohio (light red)	Observed	315	223	538	*CC Rr Dd*	
		Calculated	303	235	538		9:7
10. 22	Early Ohio (light red) × no. 4.	Observed	17	39	56	*CC Rr Dd* × *cc Rr dd*	
		Calculated	21	35	56		3:5
11	No. 41–17 (light red) × no. 4–9–1 (white).	Observed	124	225	349	*CC Rr Dd* × *cc Rr dd*	
		Calculated	131	218	349		3:5
	Selfed seedlings of family no. 11:						
11–4	No. 11–4 (faint red)		17	28	45	*Cc Rr Dd*	27:37
11–8	No. 11–8 (faint red)		7	9	16	*Cc Rr Dd*	27:37
11–3	No. 11–3 (light red)		26	10	36	*Cc RR Dd*	9:7
11–6	No. 11–6 (light red)		18	13	31	*Cc RR Dd*	9:7
11–7	No. 11–7 (light red)		31	15	46	*Cc RR Dd*	9:7
17. 26	No. 11–4 (faint red) × no. 4–27 (white).		7	11	18		
22. 26	No. 11–3 (light red) × no. 4–27 (white).		8	38	46		

TABLE 3.—*Data relating to progeny inheritance of blue cortex color*

Family no.	Parents and color	Progeny having indicated cortex color			
		Blue	Red	White	Total
		Number	*Number*	*Number*	*Number*
54. 26	Russet Rural (white) × no. 11–4 (faint red)	16	21	53	90
38. 26	Russet Rural (white) × no. 11–3 (light red)	42	52	108	202
53. 26	Russet Rural (white) × no. 41–1 (white)	0	0	31	31
40. 26	41–1 (white) × no. 11–3 (light red)	0	22	26	48

INHERITANCE OF BLUE COLOR IN THE CORTEX

Data relating to the inheritance of blue-cortex color is recorded in table 3. Blue-, red-, and white-cortex seedlings were observed in families resulting from crosses between the white-cortex Russet Rural variety and red-cortex seedlings nos. 11–3 and 11–4. The nearly equal occurrence of blue- and red-cortex individuals in each of these families suggests that the Russet Rural may have been heterozygous for a factor for blue-cortex color and that this factor, designated *P*, when present with the factors necessary for red-cortex color, produced blue-cortex color. The behavior of white-cortex seedling no. 41–1, a seedling of the Early Ohio, in crosses with seedling 11–3 and Russet Rural suggests that seedling 41–1 and Russet Rural were probably recessive for the same factor pair.

RELATION OF COLOR IN PERIDERM AND CORTEX

The individuals of family 85, progeny of a plant of unknown tuber color, are classified in table 4 in four color classes. The frequencies suggest a relation of 3:1 for periderm color and 9:7 for cortex color. A comparison of the observed frequencies with the combined ratio of 27:21:9:7 for the two character pairs show good agreement, P equaling 0.54. It appears that in family no. 85 a factor for periderm color is segregating independently of two factors for cortex color.

TABLE 4.—*Segregation of periderm and cortex color in family no. 85*

Item	Progeny having indicated color				
	Red periderm, red cortex	Red periderm, white cortex	White periderm, red cortex	White periderm, white cortex	Total
Observed	88	65	31	16	200
Calculated	84	66	28	22	200

The absence of blue periderm-red cortex and red periderm-blue cortex individuals in family no. 100.27, progeny of a cross between a blue-periderm and a red-cortex parent, as recorded in table 5, suggests that the factor P may operate in both periderm and cortex to change red color to blue. This view is in harmony with the data relating to progenies of crosses between the *Rural*, a smooth white tuber variety, and three selected parents, as recorded in table 6. Color was confined to the periderm in blue and red individuals in family no. 50.31 whose pollen parent was a red-periderm seedling. Red and blue color characterized only the cortex in those families whose pollen parents were derived from red-cortex parents. The 3:1 relation of blue to red apparent in each of these progenies suggests that the *Rural* may have been heterozygous for two duplicate factors, P and P', each capable of changing red periderm and red cortex to blue.

TABLE 5.—*Color classes in a cross of blue periderm × red cortex (accession no. 125 × 11–3–6), family no. 100.27*

Cortex color	Progeny having indicated periderm color			Total
	Blue	Red	White	
	Number	*Number*	*Number*	*Number*
Blue	5	0	4	9
Red	0	3	1	4
White	9	1	3	13
Total	14	4	8	26

TABLE 6.—*Color in periderm and cortex in progenies of crosses between the Rural variety and certain pollen parents*

Family no.	Parents and color	Progeny having color in—					
		Periderm		Cortex			Total
		Blue	Red	Blue	Red	White	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
50.31	Rural (white × no. 5-3-2 (red periderm)	20	3	0	0	30	53
56.31	Rural (white) × no. 7-1-2 (white)	0	0	26	9	26	61
58.31	Rural (white) × no 11-4-16-2 (white)	0	0	31	14	26	71

SUMMARY

This paper is concerned with the inheritance of color in periderm and cortex of the potato, *Solanum tuberosum* L.

The segregation of red and white periderm color in progenies of 11 selfed potato plants has been explained by assuming that the complementary action of factors *E*, *S*, and *F* produced red color in the periderm and that certain parents carried duplicate factors.

The inheritance of red cortex color has been explained by assuming that the complementary action of factors *C*, *R*, and *D* produced red color in the cortex.

In one family a factor for red periderm color was segregating independently of two factors for red cortex color.

Blue color in periderm and cortex has been referred to two factors, *P* and *P'*, each capable of changing red periderm and red cortex to blue.

The observations suggest that while certain factors affect color in only one tissue other factors influence color in both periderm and cortex.

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THE EFFECT OF CALCIUM CARBONATE AND SODIUM BICARBONATE ON THE TOXICITY OF GOSSYPOL[1]

By WILLIS D. GALLUP, *assistant chemist*, and RUTH REDER, *associate chemist*, *Department of Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station*

INTRODUCTION

In a previous study of the influence of certain dietary constituents on the response of rats to gossypol ingestion (*2*)[2] the authors showed that the toxicity of diets containing known amounts of gossypol was materially reduced when the diets were made basic by the addition of calcium carbonate and sodium bicarbonate. When the diets were made acidic by the addition of calcium chloride, only indirect evidence of a slight decrease in toxicity was obtained. Decreased toxicity was observed also when the protein content of the diet was increased from 13 percent to 35 percent. The conclusion was drawn that diets of high protein content and basic diets of high calcium content are favorable to the detoxication of gossypol.

Considerable importance is attached to the results since calcium and protein are variable ingredients in diets used for the bio-assay of gossypol. The importance of these constituents in feeding cottonseed products to livestock has not been fully determined, although the value of supplementary protein in cottonseed-meal rations for pigs has recently been pointed out by Robison (*5*).

In view of the favorable results obtained with sodium bicarbonate and calcium carbonate, it was deemed desirable to determine the proportion of these salts which would offer the greatest degree of protection against gossypol injury and to determine the value of each salt in the presence of moderate amounts of the other, as the principal elements of both are requisite for a normal nutritive condition. The present paper reports the results of this study. The results of other experiments, in which calcium altered the inhibiting effect of gossypol on the hydrolysis of fat by lipase, are also reported. The results of these studies are offered as further evidence of the important part played by calcium in the detoxication of gossypol.

MATERIAL AND METHODS

The manner of conducting the feeding experiments, in which albino rats were used as experimental animals, has been described in a previous paper (*2*).

The percentage composition of the basal diet to which varying amounts of sodium bicarbonate and calcium carbonate were added was as follows: Cottonseed 10, starch 49, casein 24, yeast 5, cod-liver oil 3.5, salt mixture (*3*) 3.5, and Crisco 5. The cottonseed contained 0.60 percent of gossypol. Control diets were made up with gossypol-free

[1] Received for publication Aug. 19, 1935; issued February, 1936. A preliminary report of this work was presented before the American Society of Biological Chemists in Detroit, Mich., Apr. 10–13, 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 72.

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cottonseed. Fresh diets were prepared weekly, at which time the prescribed amounts of calcium carbonate and sodium bicarbonate were added. In one experiment sodium bicarbonate was added to the basal diet at the time of feeding, and any food residues from the previous day were discarded. This procedure was followed as a precaution against the possible destruction of the gossypol, by contact with the alkali, before ingestion.

In the lipase studies the general procedure of Willstätter, Waldschmidt-Leitz, and Memmen (*7*) was followed. A glycerol extract of pig's pancreas was used as a source of lipase. The fresh tissue was dried with acetone and ether and ground in a mortar with 16 times its weight of 80-percent glycerol. After standing at room temperature for 4 hours or longer, with occasional shaking, the solution was filtered through filter paper and stored in an electric refrigerator.

Neutral olive oil was prepared by shaking a high-grade commercial oil with an equal volume of 1-percent sodium hydroxide in a separatory funnel at 60° C. After the aqueous layer had been removed, the oil was thoroughly washed with hot water and dried in the electric oven at 100° C. for 6 hours.

A 2-percent solution of egg albumin was prepared by dissolving the dry powder in an excess of ammonium hydroxide and, after this had been shaken for several hours, removing the excess ammonia with a current of warm air.

Two buffer solutions were prepared. One was composed of 66 parts of N/1 ammonium hydroxide and 134 parts of N/1 ammonium chloride and had a pH of 8.9 at 30° C. The other buffer solution was composed of equal parts of N/2 acetic acid and N/2 sodium acetate and had a pH of 4.7 at 30°.

A 1-percent solution of calcium chloride was made up with the chemically pure salt. Gossypol was prepared and purified according to methods described by Clark (*1*).

Determinations of the rate of hydrolysis of olive oil by lipase in the presence of calcium and gossypol were made in the following manner (*4*). One-half cubic centimeter of lipase extract, 3 cubic centimeters of olive oil containing various amounts of gossypol, 2 cubic centimeters of the alkaline buffer solution, 1 cubic centimeter of calcium chloride solution, and sufficient amounts of a dilute glycerol solution to keep the total volume of the water 11 cubic centimeters and the total volume of glycerol 2 cubic centimeters, were shaken together in a small Erlenmeyer flask. One cubic centimeter of egg-albumin solution was then added, and after being skaken for 3 minutes, the flask was placed in a constant-temperature water bath held at 30° C ± 0.2 for 2 hours and 57 minutes. At the end of this time the contents of the flask were washed into a 250-cubic centimeter Erlenmeyer flask with 112 cubic centimeters of 95-percent alcohol. Twenty cubic centimeters of ether was added and the fatty acids titrated with N/10 alcoholic sodium hydroxide, thymolphthalein being used as an indicator. Blank determinations were carried out without the addition of the enzyme.

Determinations in which calcium and albumin were omitted were made in the manner described above. In another series of experiments the gossypol was dissolved in the buffer instead of in the oil. In a final series of experiments hydrolysis was carried out in an acid medium with 2 cubic centimeters of the acid buffer solution replacing the alkaline buffer solution. The gossypol was dissolved in the oil.

EXPERIMENTAL RESULTS

FEEDING EXPERIMENTS WITH CALCIUM CARBONATE AND SODIUM BICARBONATE

Data obtained with rats which received diets containing 0.06 percent of gossypol and varying amounts of calcium carbonate and sodium bicarbonate, together with their controls, are shown in table 1.

Table 1 shows a conspicuous difference between the food intake of animals receiving gossypol and the intake of their controls. That of the rats on gossypol was noticeably increased by the addition of sodium bicarbonate to the diet. The same result was obtained with combinations of this salt with calcium carbonate, although the latter by itself tended to decrease the food intake of rats on gossypol diets. The average food intake of the gossypol rats on diets containing 3 percent of sodium bicarbonate slightly exceeded that of their controls, being 599 grams as compared with 585 grams. Similarly there was very little difference between the food intake of the controls and that of the gossypol rats on a diet to which a combination of 2 percent of sodium bicarbonate and 2 percent of calcium carbonate had been added. The increase in food consumption on the part of the rats on gossypol is taken as an indication of improvement in the nutritive condition of the animals.

The average gains in weight of rats on the gossypol diets likewise increased with increasing percentages of sodium bicarbonate in the diet. At the 3-percent level, sodium bicarbonate was particularly effective in producing an increase in their gains. The greatest average gain for the rats on gossypol, 118 grams, was made on a diet containing 2 percent of calcium carbonate and 2 percent of sodium bicarbonate, although the average gains made by gossypol rats on diets containing 3 percent of sodium bicarbonate or a combination of 2 percent of calcium carbonate and 1 percent of sodium bicarbonate approached this value.

TABLE 1.—*Effect of gossypol on growth of rats as influenced by the addition of various amounts of sodium bicarbonate and calcium carbonate to the diet*

Salt additions to basal diet	Gossypol content of diet	Rats	Average food consumed in 60 days [1]	Average gain in weight in 60 days [1]	Difference in gains in weight
	Percent	*Number*	*Grams*	*Grams*	*Grams*
None	0.06	12	468	88	82
	.00	7	629	170	
1 percent of $NaHCO_3$	.06	14	530	92	71
	.00	6	703	163	
2 percent of $NaHCO_3$	.06	12	527	99	50
	.00	6	659	149	
2 percent of $NaHCO_3$ [2]	.06	6	530	94	55
	.00	6	659	149	
3 percent of $NaHCO_3$	.06	7	599	106	44
	.00	7	585	150	
2 percent of $CaCO_3$	.06	6	413	87	76
	.00	6	678	163	
2 percent of $CaCO_3$; 2 percent of $NaHCO_3$	.06	7	624	118	28
	.00	6	630	146	
2 percent of $CaCO_3$; 1 percent of $NaHCO_3$	.06	9	500	102	58
	.00	9	621	160	

[1] The sum of the averages obtained with males and females divided by 2.

[2] 2 percent of sodium bicarbonate added daily to gossypol diet. The figures presented for the controls in this group are those given for the controls in the preceding group.

An important point brought out in table 1 is that the addition of 2 percent of calcium carbonate by itself effected no improvement in

the growth of the gossypol or control rats; its addition in combination with 1 percent or 2 percent of sodium bicarbonate produced a noticeable improvement in the gains made by the gossypol rats, increasing their average gain from 88 grams to 102 and 118 grams. This combination of salts was likewise more effective than single additions of 1 percent or 2 percent of sodium bicarbonate.

It should be noted that in the case of the control rats the addition of sodium bicarbonate, especially in amounts exceeding 1 percent, either alone or in combination with calcium, was unfavorable to gains in weight and in one instance (3 percent of sodium bicarbonate) caused decreased food consumption. Since the opposite effect was produced in the case of the rats on gossypol, the difference between gains made by control and gossypol rats on diets containing over 1 percent of sodium bicarbonate becomes small, being 55 grams or less, as compared with 82 grams. One criterion used to judge the effectiveness of each dietary alteration is that the effect so produced be more apparent in the performance of the rats on gossypol than in their controls. It seems reasonable therefore to believe that by the addition of such a readily soluble base as sodium bicarbonate conditions are produced that are favorable to the detoxication of gossypol although unfavorable to the growth of animals on a normal diet.

In order to facilitate a comparison of the gains made by gossypol rats on what appeared to be the least effective combinations of salts with those that received the most effective combinations, the animals have been divided into three groups. Group 1 includes the rats which, with respect to salt additions, received the following three diets: No addition, 1 percent of sodium bicarbonate and 2 percent of calcium carbonate. These made average gains between 87 and 92 grams. Group 2 consists of those which received 2 percent of sodium bicarbonate. In group 3 are those which received 3 percent of sodium bicarbonate and the two combinations of sodium bicarbonate and calcium carbonate. These made average gains between 102 and 118 grams. The average gain in weight made by the rats that received 2 percent of sodium bicarbonate falls between the average gains in weight of the rats in the first and third groups. The average gains and standard deviations of the means for males and females in these three groups are given in table 2. The formula used for the standard deviation was

$$\sigma = \sqrt{\frac{\Sigma d^2}{N}}$$

in which

σ = standard deviation of the average,
Σd^2 = sum of the deviations from the average, squared, and
N = number of observations.

Table 2 shows the beneficial effect of increased amounts of calcium carbonate and sodium bicarbonate in diets containing gossypol. Although it is not possible to make a sharp distinction between the different amounts and combinations of these salts with respect to their detoxicating properties, the amounts added to the diets of group 3 were undoubtedly more effective than the single additions to the diets of group 1. This is more apparent in the average growth of females than of males. That this result was not due to the increased

nutritive value of the diets is shown by the performance of the control rats.

TABLE 2.—*Gains in weight made by 3 groups of rats on gossypol and gossypol-free diets*

Sex of rats and groups [1] compared	60-day gains made by rats on—									
	Gossypol diets					Gossypol-free diets				
	Rats	Minimum gain	Maximum gain	Average gain	Standard deviation	Rats	Minimum gain	Maximum gain	Average gain	Standard deviation
Males:	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Number*	*Grams*	*Grams*	*Grams*	*Grams*
Group 1	18	75	141	109	19.92	7	180	217	202	11.09
Group 2	10	77	166	108	27.00	3	169	182	175	----
Group 3	15	77	167	125	28.78	13	155	217	186	17.61
Females:										
Group 1	12	41	115	72	17.55	12	102	153	129	13.71
Group 2	8	63	114	87	15.26	3	100	137	123	----
Group 3	8	69	108	91	13.27	9	112	125	120	4.58

[1] Group 1 includes rats which, with respect to salt additions, received the following 3 diets: No addition, 1 percent of $NaHCO_3$, and 2 percent of $CaCO_3$; group 2 includes rats which received 2 percent of $NaHCO_3$; group 3 includes rats which, with respect to salt additions, received the following 3 diets: 3 percent of $NaHCO_3$, 2 percent of $CaCO_3$+2 percent of $NaHCO_3$, and 2 percent of $CaCO_3$+1 percent of $NaHCO_3$.

LIPASE STUDIES

The effect of increasing amounts of gossypol on the hydrolysis of fat by lipase, both alone and in combination with calcium and albumin is shown in table 3. In experiment 25, which was carried out in a system with an initial pH of 8.9, the inhibition of hydrolysis produced by 8 milligrams of gossypol dissolved in the oil amounted to 63 percent. In the presence of calcium, a substance which normally activates the reaction, hydrolysis was practically stopped by 8 milligrams of gossypol. The same result was obtained when both activators, calcium and albumin, were present.

In experiment 26, in which another enzyme preparation was used gossypol was dissolved in the buffer solution. In the absence of activators, a maximum inhibition of 58.6 percent was produced by 8 milligrams of gossypol. With albumin as an activator, the amount of inhibition was slightly increased by 12 milligrams of gossypol. In the presence of calcium, however, this amount of gossypol practically stopped hydrolysis.

Experiment 28 was conducted in a system with an initial pH of 4.7. This was secured by replacing the alkaline buffer solution with an acetate buffer solution. In this system, albumin inhibited the hydrolysis, and calcium was without appreciable effect. The amount of inhibition produced by 12 milligrams of gossypol dissolved in the oil was approximately the same as that in experiment 25, being 71.8 percent. Gossypol in the presence of calcium did not completely prevent hydrolysis, as it did in the presence of the alkaline medium.

The correct interpretation of these results based upon proven facts must necessarily await more complete knowledge of the action of both lipase and gossypol Three important facts have been established: (1) Gossypol inhibits lipase activity in basic and acidic media; (2) complete inhibition by gossypol in an alkaline medium is dependent upon the presence of calcium, and in an acid medium the degree of

inhibition is independent of the calcium; (3) calcium precipitates gossypol from an alkaline solution (*2*).

TABLE 3.—*Effect of gossypol on lipase action both alone and in combination with calcium and albumin in basic and acidic media as shown in 3 experiments*

[Period of hydrolysis, 3 hours; temperature, 30° C.]

EXPERIMENT 25; ENZYME 4; GOSSYPOL DISSOLVED IN OIL; INITIAL pH, 8.9

Albumin (milligrams)	Calcium chloride	Gossypol	Hydrolysis in terms of N/10 NaOH used in titration	Inhibition in terms of N/10 NaOH used in titration	Inhibition	Albumin (milligrams)	Calcium chloride	Gossypol	Hydrolysis in terms of N/10 NaOH used in titration	Inhibition in terms of N/10 NaOH used in titration	Inhibition
	Milligrams	*Milligrams*	*Cubic centimeters*	*Cubic centimeters*	*Percent*		*Milligrams*	*Milligrams*	*Cubic centimeters*	*Cubic centimeters*	*Percent*
0	0	0	7.3			20	10	0	21.4		
		4	3.9	3.4	46.6			4	15.5	5.9	27.6
		8	2.7	4.6	63.0			6	1.2	20.2	94.4
		12	2.1	5.2	71.0			8	.8	20.6	96.3
0	10	0	20.0					10	.8	20.6	96.3
		4	10.3	9.7	48.5			12	.8	20.6	96.3
		8	1.0	19.0	95.0						
		12	.6	19.4	97.0						

EXPERIMENT 26; ENZYME 5; GOSSYPOL DISSOLVED IN BUFFER; INITIAL pH, 8.9

0	0	0	7.0			0	10	0	25.0		
		2	7.0	0	0			2	19.4	5.6	22.4
		4	5.0	2.0	28.6			4	12.2	12.8	51.2
		8	2.9	4.1	58.6			8	4.4	20.6	82.4
		12	2.9	4.1	58.6			12	.8	24.2	96.8
20	0	0	15.0								
		2	13.1	1.9	12.7						
		4	11.3	3.7	24.7						
		8	5.8	9.2	61.3						
		12	5.0	10.0	66.6						

EXPERIMENT 28; ENZYME 5; GOSSYPOL DISSOLVED IN OIL; INITIAL pH, 4.7

0	0	0	8.5			0	10	0	8.6		
		2	3.7	4.8	56.5			2	3.9	4.7	54.7
		4	3.2	5.3	62.4			4	3.2	5.4	62.8
		8	3.0	5.5	64.7			8	2.4	6.2	72.1
		12	2.4	6.1	71.8			12	2.2	6.4	74.4
20	0	0	2.5								
		2	1.7	.8	32.0						
		4	1.5	1.0	40.0						
		8	1.4	1.1	44.0						
		12	1.2	1.3	52.0						

Waldschmidt-Leitz (*6*) has explained the activating effect of calcium on lipolysis as being due to "the production of colloid particles which exert an absorbent action with respect to the enzyme as well as to the substrate and thereby facilitate the reaction." Gossypol, by its affinity for calcium in alkaline solution, apparently prevents the formation of this "coupled absorbent" [3] or, as seems more likely, completely removes it from its sphere of activity.

The relationship of these observations to the results of the feeding experiments is discussed later.

[3] A "coupled absorbent" involving calcium and albumin has been represented by Willstätter et al. (*7, p. 100*) as calcium oleate—albumin
fat lipase

DISCUSSION

The foregoing feeding experiments have demonstrated the beneficial effect of sodium bicarbonate and calcium carbonate on the growth of rats receiving toxic diets which contained 0.06 percent of gossypol. The increased growth of the rats which received 3 percent of sodium bicarbonate or combinations of 1 and 2 percent of sodium bicarbonate with 2 percent of calcium carbonate was shown to be due to the action of these salts on gossypol since no corresponding increase in growth rate was produced by the addition of these salts to a gossypol-free diet of the same composition. In fact sodium bicarbonate in amounts exceeding 1 percent proved detrimental to the growth of rats on gossypol-free diets. That the beneficial effect of these salt additions was not due merely to an increase in potential alkalinity of the diets is shown in the poor performance of the animals that received 2 percent of calcium carbonate. In other experiments (unpublished) 3 percent of calcium carbonate has likewise been found to be ineffective.

The value of 3 percent of sodium bicarbonate in gossypol diets is attributed to the solubility of this salt with the production of an alkaline medium in which gossypol is very reactive and unstable. Conditions are thereby made favorable for the reaction of gossypol with calcium and possibly other constituents normally present in the diet (*2*). Iron salts are particularly efficacious detoxicants.

The inability of calcium salts to detoxicate gossypol in the absence of sodium bicarbonate was in all probability due to an unfavorable reaction medium. When dissolved in dilute alkali, gossypol is slowly precipitated by calcium salts.

The activity of gossypol in an alkaline medium and its probable reaction with calcium was again indicated in a study of the effect of gossypol on the hydrolysis of fat by lipase. Gossypol inhibited the hydrolysis in both acidic and basic media. In an acid medium the degree of inhibition produced by gossypol was unaffected by the presence of calcium. In an alkaline medium, inhibition by gossypol was altered by calcium; and, despite the activating action of calcium, hydrolysis practically stopped under these conditions. Apparently a reaction took place between calcium and gossypol which involved the enzyme. A reaction between gossypol and the activator-enzyme complex of which calcium is a part seems probable.

SUMMARY

The effect of calcium carbonate and sodium bicarbonate on the toxicity of gossypol diets was determined by feeding experiments with young rats. The experimental diets were made up with 10 percent of cottonseed which contained 0.6 percent of gossypol. Calcium carbonate and sodium bicarbonate were added to the diets in varying proportions and amounts. Similar additions were made to control diets made up with gossypol-free cottonseed. The criterion used to judge the effectiveness of these salt additions in modifying the deleterious action of gossypol was that the effect so produced be more apparent in the growth of the rats on gossypol diets than in that of their controls.

The best growth of the rats receiving gossypol was obtained with a diet to which 2 percent of calcium carbonate and 2 percent of sodium bicarbonate had been added. Next in value were diets to which 3 percent of sodium bicarbonate or a combination of 2 percent of calcium carbonate and 1 percent of sodium bicarbonate had been added. It was evident from the performance of the control rats that the beneficial effect of these salts was due to their action on gossypol and not merely to their influence on the nutritive properties of the basal diet.

The value of sodium bicarbonate in conjunction with calcium carbonate or in the presence of normal amounts of dietary calcium, was attributed to its solubility with the production of an alkaline medium in which gossypol is unstable and susceptible of precipitation by calcium. Support for this belief was found in a study of the effect of gossypol on the hydrolysis of fat by lipase, a process which when initiated in an alkaline medium, is activated by calcium.

In a system with an initial pH of 4.7 (acid region) 12 milligrams of gossypol inhibited but failed to prevent hydrolysis. This result was obtained both in the presence of calcium and in its absence. In a similar system with an initial pH of 8.9 (alkaline region) gossypol, in the absence of calcium, only inhibited hydrolysis; whereas in the presence of calcium, 8 milligrams of gossypol almost entirely prevented hydrolysis.

The conclusion is drawn that the action of calcium carbonate and sodium bicarbonate in gossypol diets is one of detoxication, involving a reaction between gossypol and calcium in an alkaline medium.

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APPARATUS AND PROCEDURE FOR SEPARATING COTTON ROOT ROT SCLEROTIA FROM SOIL SAMPLES [1]

By C. H. ROGERS

Plant pathologist, Texas Agricultural Experiment Station

INTRODUCTION

In field studies of rotation, cultural, and soil-amendment practices in relation to the control of the cotton root rot disease, caused by *Phymatotrichum omnivorum* (Shear) Duggar, the number or percentage of dead and dying plants and crop yields has been used as a measure of the effectiveness of the treatment. How the causal organism is affected by such practices has received little or no attention.

The disease is perpetuated by sclerotia or resting bodies and infected roots with superficial or closely associated mycelial strands. In clean fallows and fields planted to nonsusceptible crops, if no infected roots of susceptible plants are present, sclerotia are the only means of carry-over. In the black Houston clay soils and other soil types of central Texas the sclerotia are brown in color when mature and vary from less than a millimeter to a centimeter or more in size. The bulk of sclerotia are found at soil depths of between 8 and 36 inches. Inasmuch as sclerotia may be present in large quantities and may remain viable for a number of years, it is important to know how any control treatment affects their viability and dissipation.

To study sclerotia in the laboratory it is necessary to separate them from the soil. Because of their small size it is impractical to separate by hand during a season or part of a season sufficient samples to be of much value. Washing in screen flats with a garden hose, as reported by various workers [2] [3] [4] is a method applicable only to pot or other small samples. By means of the apparatus and methods here described, from 10 to 15 barrel samples of soil, weighing from 2 to 4 tons may be handled, and the sclerotial separation completed in a single day.

DESCRIPTION OF APPARATUS AND WASHING PROCEDURE

The machine used in the initial separation of sclerotia and soil is shown in figure 1. The essential parts of the machine are the frame, the cradlelike hoisting or tilting and feeding device, a vertically mounted, coarse-mesh cylinder screen with agitator, a fine-mesh horizontal screen below the cylindrical screen, and a splash-drain vat below and surrounding the lower screen.

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[1] Received for publication Aug. 9, 1935; issued February, 1936. Contribution no. 336, Technical Series, Texas Agr. Expt. Sta.

[2] KING, C. J., and HOPE, C. DISTRIBUTION OF THE COTTON ROOT-ROT FUNGUS IN SOIL AND IN PLANT TISSUES IN RELATION TO CONTROL BY DISINFECTANTS. Jour. Agr. Research 45: 725–740, illus. 1932.

[3] ———, HOPE, C., and EATON, E. D. THE COTTON ROOT-ROT FUNGUS INDIGENOUS IN ARIZONA DESERTS. Science (n. s.) 75: 48–49. 1932.

[4] TAUBENHAUS, J. J., and EZEKIEL, W. N. METHODS OF SAMPLING FOR SCLEROTIA. Tex. Agr. Expt. Sta. Ann. Rept. 45: 60–61. 1932.

The frame proper is made of ⅜ by 2 by 2 inch angle iron, braced with ⅜-inch steel plate. All the joints are welded. At the base, the machine is 32 by 32 inches, tapering to a smaller dimension at the top.

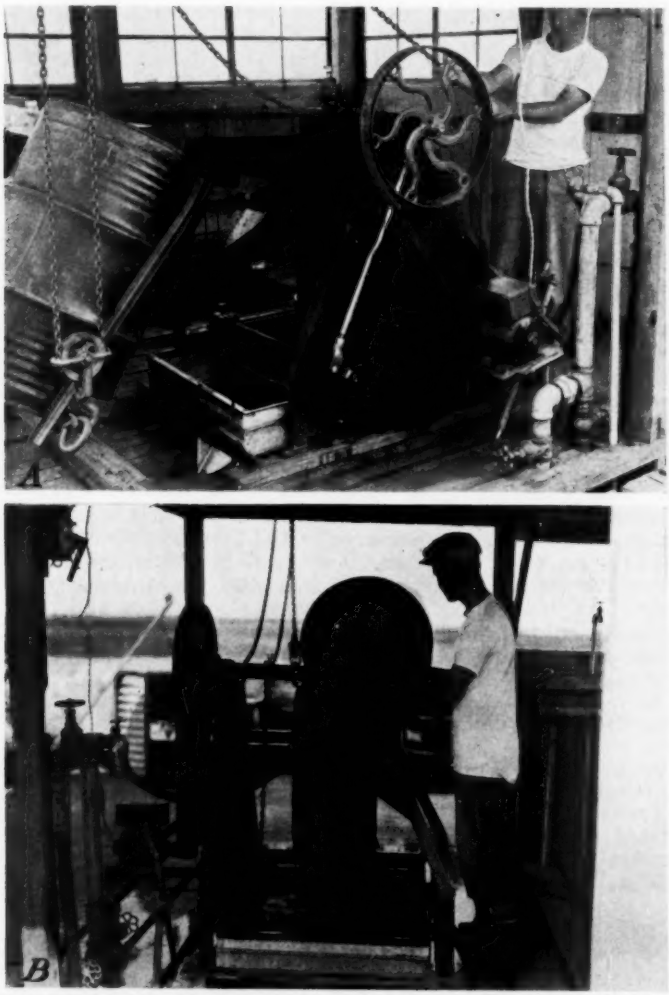

FIGURE 1.—Machine used in separating root rot sclerotia from soil: *A*, Side view, showing barrel-hoisting and tilting-frame motor, eccentric shaft (*a*), and adjustable overflow (*b*); *B*, front view of machine in operation. The soil from the barrel is washed directly into the cylinder screen.

It is approximately 40 inches high. A bowllike chute, made of ¼-inch plate, is welded around the top of the machine and tapers downward to the upper edge of the vertical screen cylinder. It is made wide enough for samples to be poured in or washed from a barrel

without any waste. The machine is driven by a small electric motor (one-third horsepower), seated on a swinging base. The large pulley used on the main drive shaft reduces its speed to approximately 250 revolutions per minute. The motor and belt are protected from splashing water by a panel of 18-gage iron bolted to the side of the frame adjacent to the motor. The main shaft and the eccentric and agitator shafts, mentioned later, are equipped with ball bearings, lubricated through grease cups.

Inasmuch as barrel-size samples are usually used in this work, a hoisting and tilting frame is attached to facilitate handling. It is made of the same material as the frame of the machine, and is constructed of such a height that the upper edge of the barrel extends about 6 inches over the chute around the top of the vertical screen. The top of the frame is hinged from the edge of the chute. A half-ton chain hoist attached to a rafter provides for the necessary lifting power. Oil drums of uniform height are used for sample containers.

Because of the puddling and the highly colloidial nature of the Houston soils, it is necessary to use a rather large quantity of water and thorough agitation to screen the material rapidly. The sample is first washed through the coarse cylinder screen and drops onto a finer screen below.

This cylinder screen is 12 inches in diameter, and 15 inches high, and is made of 8 by 8 mesh (8 meshes per linear inch) no. 16 (Washburn & Moen gage) iron wire. The openings and wire are each one-sixteenth of an inch in diameter. It is necessary to use a heavy wire, because of the harsh action of shell and pieces of plant stems or occasional small rocks that are present in samples of soil. The agitator in this screen cylinder is made of 1½-inch shafting with small rods inserted through the lower 15 inches of the shaft in a staggered manner. It is rigidly supported at the top with ball bearings and is operated from the pulley or main shaft by beveled gears. The size ratio of the two gears is such that the speed of the agitator is reduced to 150 r. p. m. Bearings and gears are enclosed to retain oil and protect them from silt and water.

To keep the sample in suspension as much as possible while agitation and screening are taking place, the water is supplied through numerous vents in a removable compartment at the bottom of this screen. This water-inlet compartment is shown partially removed in figure 2, *A*. It is also made of ⅜-inch steel plate, with vents one thirty-second of an inch in diameter spaced about 1 inch apart over the top surface. A connecting hose supplies water under enough pressure to eject streams upward 2 or 3 feet through the small vents. The inlet compartment is held snugly against the bottom of the cylinder by a grooved flange support. Since the greater number of the sclerotia pass through this screen, it is necessary to pass the material on to a finer screen.

The horizontal screen, upon which the washings fall, is 36 inches long, 24 inches wide, and 4 inches deep. It is made of 35-mesh no. 29 (Washburn & Moen gage) brass-wire cloth with openings of 0.0136 inch. No. 12 sheet steel is used for the framework for this wire. Screen surfaces are provided at each end of the screen as well as the bottom, but the sides are solid (fig. 2, *B*). A reciprocating motion is provided for the screen by an eccentric shaft (fig. 1, *A*, *a*).

The length of screen stroke is varied by changing the position of the attachment on the lower end of the eccentric shaft on the arm of the screen-propelling shaft. A stroke of 2 inches is most satisfactory.

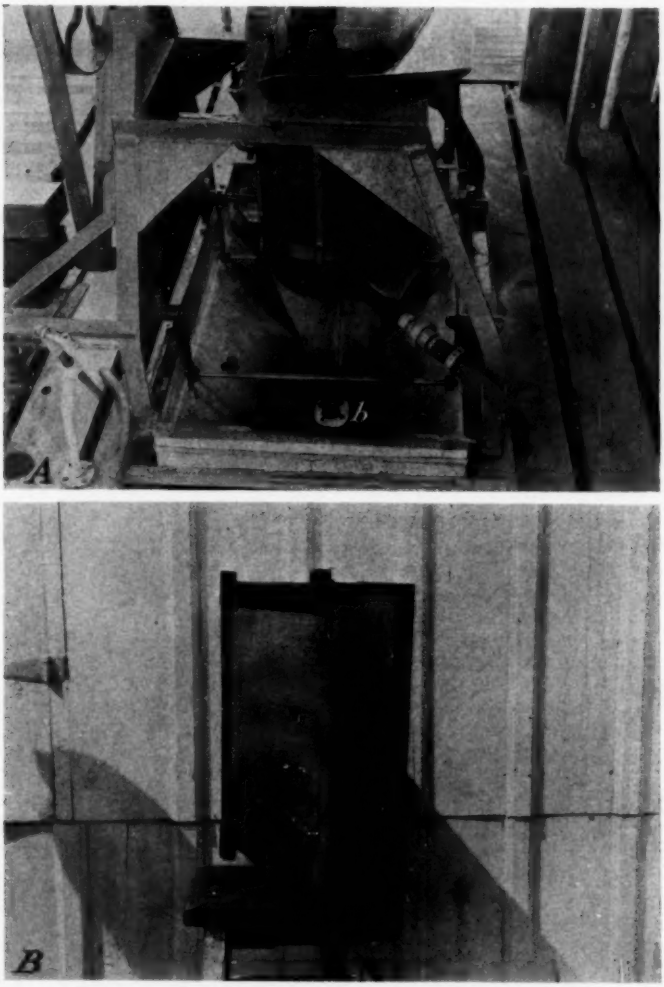

FIGURE 2.—*A*, Close view of part of the machine showing cylinder screen, water inlet and hose, horizontal screen supporting shafts (*a*, *a*), and splash-drain vat (*b*); *B*, horizontal fine-mesh screen.

The screen is supported by the two shafts shown in figure 2, *A*, *a*, *a*, and is held in place by four pieces of U iron, welded on the bottom of the screen frame. To withstand the grinding action of silt and water, these pieces of U iron are lined with a hard steel alloy. The

areas on the shafts where these bearings rest are coated with the same material. To remove the screen, it is necessary only to lift it from these shafts. These two shafts are suspended by arms at each end from 4-inch plain bearings, each supplied with a grease cup. The rear shaft and arms hang loose, but the forward, or propelling, arms and shaft, are rigidly connected to the eccentric. A rubber hood is tied over each of the bearings to preserve them against silt and water.

To aid screening on the fine-mesh screen and facilitate proper drainage, a pyramid-shaped vat is supported around and below the screen. Water and silt from the vat are drained through a 2-inch drainpipe. While in operation, the water level in this vat is kept only about half an inch above the bottom of the screen by an adjustable overflow in the wall of the vat (fig. 1, *A*, *b*). By keeping the water at this low level, screening is not only accelerated, but splashing, caused by the movement of the screen, is reduced to a minimum. A valve in the drainpipe is controlled from above the floor by an extension handle, shown projecting just below the motor in figures 1, *A*, and 2, *A*. This valve is left partially open during screening to allow silt to move out freely. The vat is quickly filled or emptied by closing or opening the valve.

In screening a sample of soil, the sample is either emptied by hand or washed from a barrel into the cylinder screen. The finer particles, and most of the sclerotia, are washed through the cylinder onto the horizontal screen below. When screening is complete, the water inlet, at the base of the cylinder screen, is partly or entirely slid from its flange support and the residue dumped or washed onto the lower screen. The lower screen is then removed and the total residue of both screens washed into a suitable container. There is usually about half a gallon to a gallon of this residue left from a barrel of Houston soil. It is composed primarily of shell and bits of organic matter and any sclerotia that might be present.

RELATION OF RETENTION OF SCLEROTIA TO SIZE OF SCREEN

To determine just how fine a screen should be used in order to retain all sclerotia in screening procedures, a number of screens of different-size meshes have been used. The finest wire previously used was a 20-mesh per inch, similar to ordinary window screen. In a series of tests, an 18-mesh iron wire was used on the cylinder, a 40-mesh brass wire on the lower screen, and 60-mesh brass wire was placed under the outlets of the drain and overflow pipes. These tests showed that an average of 70 percent of the sclerotia passed the 18-mesh wire but that none passed the 40-mesh wire, as is shown by the fact that none was caught on the 60-mesh wire. The 18-mesh wire on the cylinder screen has since been changed to the heavier 8 by 8 mesh, and the wire on the horizontal screen has been changed from the 40-mesh to the 35-mesh. This 35-mesh screen is made of heavier wire than the 40-mesh and has approximately the same-size openings. Rea [5] found, in a number of tests, that 80 percent of the sclerotia passed a 16-mesh brass wire and 7 percent passed a 30-mesh wire. The openings in this 30-mesh wire were 0.021 inch and were considerably finer than any previously reported as being used for this work.

[5] REA, H. E. RECLAIMING COTTON ROOT ROT SCLEROTIA FROM SOIL SAMPLES. Tex. Agr. Expt. Sta., Substa. 5, Ann. Rept. 1933. [Not published.]

SEPARATION OF SCLEROTIA FROM SCREEN RESIDUE

To further reduce the amount of material from which sclerotia must be picked, the residue is stirred with a sugar solution of specific gravity of 1.15 to 1.25, or about 110 to 150 parts of sugar to 100 parts of water. This allows the sclerotia, which are slightly heavier than water, to float, while the heavier shell and gravel sink. The sclerotia, along with other light organic matter, are then decanted off. To assure the removal of all the sclerotia, this procedure is repeated twice. A few other materials were tried for this purpose. Sugar was found to be the best, however, because it is not expensive, is not toxic to sclerotia, and is readily obtainable. The sugar solution is kept at the desired specific gravity by testing with a hydrometer and adding sugar when necessary. When sclerotia are to be separated with the sugar solution, the material in the container is allowed to stand a few seconds after being stirred before the decanting is begun. The sclerotia and residue are then decanted onto a fine-mesh strainer and washed thoroughly under the tap to remove all sugar. In figure 3 are shown the fractions in the separation procedure. The three dishes at the right (*B*, *C*, *D*) contain the total amount of screen residue from the barrel of soil at the left. The two Petri dishes (*C*, *D*) contain the organic matter and sclerotia separated from the screen residue by using the sugar solution. The Petri dish (*D*) at the right contains the sclerotia (approximately 200) picked by hand with forceps from the organic matter in the other Petri dish.

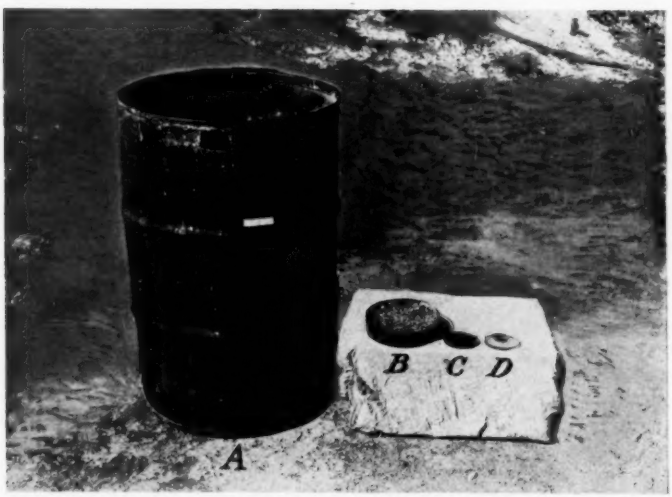

FIGURE 3.—Screen residue left after washing a barrel of soil (*A*); sclerotia (*D*) and rubbish (*C*) have been separated from shell and gravel (*B*) with sugar solution.

A number of tests were made to determine whether the sugar used in this procedure had any effect on the germination of sclerotia. In these tests, quantities of sclerotia were picked from six different samples, after they had been screened and just before they were

treated with sugar, and compared with sclerotia separated from the same samples with the sugar solution. There was very little difference in the germination of the sclerotia separated by the two methods. The average germination of those removed by hand was 52 percent and that of those separated with the sugar solution, 54 percent.

SUMMARY

The sclerotial stage of the cotton root rot fungus *Phymatotrichum omnivorum* (Shear) Duggar is one of the most important means of perpetuating the root rot disease in Texas.

Sclerotia in the Houston clay soils of central Texas vary in size from less than a millimeter to a centimeter or more.

Apparatus and procedures for the rapid mechanical separation of sclerotia from soil samples are described.

In the initial machine separation, the soil is reduced to a finely divided state by the aid of water and a rotary agitator in a coarse-mesh cylinder screen. The material passes onto a finer screen partially submerged in water and moving in a reciprocating manner. As much as one-half gallon to a gallon of residue consisting of shell, gravel, and sclerotia remain in both screens when a barrel-size sample of soil is mechanically separated. The sclerotia are floated from the heavier shell and gravel residue by stirring in a sugar solution with a specific gravity of 1.15 to 1.25. The sugar solution does not affect the viability of the sclerotia.

U. S. GOVERNMENT PRINTING OFFICE: 1936